Review

Autosomal dominant polycystic kidney disease: pathophysiology and treatment

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Introduction

Autosomal dominant polycystic disease (ADPKD) is one of the most common monogenic disorders, and globally is the third most common cause of end-stage kidney disease. Until recently, the causes of this disease remained obscure. However, the past decade has seen enormous advances in the understanding of the pathophysiology and genetics of this condition, and recent studies have suggested the possibility of specific treatment for slowing cyst growth. This review attempts to address three central questions: (i) how cysts are created; (ii) how mutated gene products give rise to cysts; and (iii) the prospects for treatment.

Cyst creation

Cysts usually develop from the collecting duct, and are highly focal in nature, only developing in a very small proportion of nephrons. They generally increase in size and number throughout life, and encroach on normal renal tissue, causing macrophage infiltration, neovascularization, progressive fibrosis and a slow deterioration in renal function. The disease only reaches the stage of chronic renal failure or end-stage renal disease in the fourth or fifth decade in the case of autosomal dominant polycystic disease type 1 (PKD1), or a decade later in the case of autosomal dominant polycystic disease type 2 (PKD2), in approximately 50% of patients.

Cysts are originally connected to the mother tubule, but eventually the connection closes off, and the cysts can then only enlarge by a process of increased proliferation of mural epithelial cells and tubular secretion. In ADPKD, these cells undergo a remarkable phenotypic change, from the usual non-proliferative and reabsorptive phenotype of collecting duct principal cells, to a secretory and proliferative phenotype; this process has been extensively investigated by Grantham and coworkers. They initially microdissected cysts from polycystic kidneys, and suspended them in a hydrated collagen gel. The growth of the cysts could then be measured by microscopy, and the physiology of transport in the cyst cells studied by the use of agonists or antagonists of transporters.

Since cysts usually originate from collecting ducts, the prototype cell involved is considered to be the cortical collecting duct (CCD) principal cell, the normal phenotype of which is shown in Figure 1a. These cells normally reabsorb NaCl: the sodium enters the luminal membrane via the epithelial sodium channels (ENaC); the chloride ions via chloride channels. The driving force is the low intracellular sodium concentration caused by the basolateral Na\(^+\)-K\(^+\)-ATPase. Potassium ions exit the luminal membrane via Renal Outer Medullary K\(^+\) (ROMK) channels driven mainly by the negative lumen potential caused by the inwardly directed sodium current. Water also enters these cells across the luminal membrane via vasopressin-sensitive Aquaporin 2 channels (not shown).

Cystic epithelial cells are markedly different from normal CCD principal cells (Figure 1b).
Vasopressin and cyclic AMP are potent cystogens, dramatically increasing cyst size and stimulating transepithelial fluid secretion. If ouabain, the Na\(^+\)-K\(^+\)-ATPase inhibitor, or furosemide, the Na\(^+\)-2Cl\(^-\)/C\(_10\)-K\(^+\) (NKCC2) co-transporter inhibitor, are added to the cyst interior, they have no effect on cyst growth, but if they are added to the exterior of the cyst, growth is inhibited,\(^6\) suggesting that cell growth is dependent on the activity of basolateral Na\(^+\)-K\(^+\)-ATPase and NKCC2. The basolateral membrane is the normal location of the Na\(^+\)-K\(^+\)-ATPase, but NKCC2 also appeared to be present on this membrane. NKCC2 is the luminal Na\(^+\),K\(^+\) and Cl\(^-\)/C\(_10\) transporter of the thick ascending limb of Henle, and is not constitutively active in CCD cells. Na\(^+\) and Cl\(^-\) are the main solutes secreted with the fluid, dependent upon a secondary active transport of chloride from the blood into the urine. Chloride ions enter the epithelial cells via the NKCC2 co-transporter in the basolateral membrane, and are then extruded from the luminal membrane into the cyst lumen via chloride channels.\(^8\)–\(^10\)

The luminal chloride channels appear to be of two types: (i) cystic fibrosis conductor regulator (CFTR) channels,\(^11\) and (ii) purinergic chloride channels, driven by ATP secreted into the cyst lumen.\(^12\) This powerful chloride secretion creates a negative luminal potential, enabling a passive paracellular flux of sodium ions into the cyst. The epithelial sodium channel, ENaC, usually responsible for sodium reabsorption across the collecting duct principle cell luminal membrane, appears to be suppressed by expression of CFTR, thus facilitating net chloride secretion into the cyst lumen.\(^9\) The secretion of sodium chloride then causes an osmotically-driven water flux. Thus, sodium chloride and then water are secreted into the lumen of the cyst,\(^6\) in remarkable contrast to the functioning of normal CCD cells (Figure 1b).

Cyclic AMP thus plays a central role in the fluid secretion in cysts.\(^2\),\(^13\) The other vital process in cyst expansion is increased cellular proliferation, and this begs the question as to whether cAMP is also implicated in increased cell growth in ADPKD. Cyclic AMP is anti-mitotic in normal kidney,\(^7\) but stimulates proliferation of mural epithelial cells from ADPKD cysts.\(^14\) Mitogen epidermal growth factor (EGF) activates this pathway via receptor tyrosine kinase, Raf-1, mitogen-activated kinase (MEK-1), and extracellular-regulated protein kinase (ERK). cAMP activates B-Raf, a kinase normally quiescent in the tubular epithelium, and this increases the activation of ERK, leading to increased cell division.\(^15\) In a recent study,\(^16\) intracellular Ca\(^2+\) levels were measured in normal human kidney (NHK) cells and in epithelial cystic cells from ADPKD. Steady state levels were 20 nM lower in ADPKD cells than in NHK cells. Treatment of ADPKD cells with a Ca\(^2+\) channel activator or with a Ca\(^2+\) ionophore caused sustained increases in intracellular Ca\(^2+\) and completely reversed the mitogenic response to cAMP. This elevation in intracellular Ca\(^2+\) blocked cAMP-dependent B-Raf and ERK activation. Thus the mitogenic effect of cAMP in ADPKD appears to be mediated via a lower intracellular Ca\(^2+\), due to lack of normal PKD1 or PKD2 gene products (see below). Cyclic AMP in PKD thus causes enlargement of cysts via two mechanisms: (i) stimulating secretion of fluid into the cyst lumen; and (ii) stimulating growth of the epithelial cells lining the cyst.
How do the mutated cell products give rise to cysts?

About 86% of cases of ADPKD are caused by a mutation of the *PKD1* gene on chromosome 16; most of the remainder are caused by mutations of the *PKD2* gene on chromosome 4. These two diseases are phenotypically almost identical, differing only by the higher age of diagnosis with PKD2, and its slower progression of to end-stage renal disease.

The product of *PKD1*, polycystin-1 (PC-1), is a very large protein (4300 amino acids), and is a membrane glycoprotein widely expressed in epithelial cells. It is also expressed in tight junctions, adherens junctions, desmosomes, apical junctions and primary cilia. Polycystin-2 (PC-2), the product of *PKD2*, is a smaller protein (968 amino acids) mainly present in the endoplasmic reticulum membrane, but also in the cell plasma membrane. PC-1 and -2 are joined via a domain in the carboxy-tail of PC-1, and appear to act in concert.

Secondly, PC-1 can also cause entry of extracellular Ca\(^{2+}\) via cell membrane PC-2. Thirdly, PC-1 activates heterotrimeric G-proteins, thus affecting activity of adenyl cyclase, MAP kinases and other effectors that can effect fluid secretion, proliferation and differentiation. However, the interaction of PC-2 with the coiled-coil domain of PC-1 inhibits G-protein signalling. Lastly, PC-1 can induce cell cycle arrest via activation of the JAK-STAT signaling pathway. In summary, activation of PC-1 and -2 appears to cause an increase in intracellular Ca\(^{2+}\) levels, causing a reduction in cAMP via direct inhibition of cAMP and stimulation of phosphodiesterase activity, which metabolizes cAMP. They also have an anti-mitotic effect, causing cell cycle arrest.

Shillingford *et al.* have recently shown that the PC-1 tail reacts with tuberin, the product of the *TSC2* gene. Mutations of this gene lead to tuberous sclerosis, a much less common disorder than ADPKD, but which is also associated with renal cysts. The *PKD1* and *TSC2* genes are located close together, and patients with large chromosome deletions affecting both genes suffer from severe, early onset polycystic kidney disease. The finding that tuberin interacts with the PC-1 tail could have important consequences: tuberin inactivates the Ser/Thr kinase mTOR (mammalian Target Of Rapamycin), whose activity has been linked to increased cell growth, apoptosis and alterations in differentiation.

Cyst-lining cells in ADPKD show very high mTOR activity, and mTOR may be part of the PC-1/tuberin complex. Thus, PC-1 may normally suppress mTOR activity via tuberin, and mutations of PC-1 may lead to aberrant mTOR activation, resulting in abnormal growth, proliferation and de-differentiation of tubular epithelial cells, resulting in cysts. mTOR, as its name indicates, is inhibited by the immunosuppressant drug rapamycin. Shillingford *et al.* therefore treated two different polycystic mouse models with rapamycin, and found a dramatic regression in kidney size in both models. In addition, they identified a group of renal transplant patients who suffered from ADPKD, and who were receiving rapamycin as part of their immunosuppressive regimen. They were able to show a reduction in kidney volumes in these patients of 25% over a period of 2 years, as measured by MRI. These findings may have important therapeutic potential (see below).

Another fascinating recent finding has been the connection between PC-1 and -2 and primary cilia.

Although the existence of primary cilia has been known for over 100 years, their function has not been clear. All renal epithelial cells (except for collecting duct intercalated cells) possess a single primary cilium. Primary cilium contains a number of unique proteins which are critical for the normal function of these organelles, including polaris, kinesin, dinein and cystin. They also contain PC-1 and -2. Disruption of a number of these proteins causes abnormalities of ciliary structure or function, and various forms of cystic disease, including ADPKD. Much evidence for this has come from animal models of cystic disease. Thus in the mouse *orpk* model of PKD, in which the ciliary protein polaris is mutated, mice null for this gene had absent or stunted primary cilia.

A kidney-specific conditional knockout disrupting a ciliary motor unit, *kif3a*, in tubular epithelial cells resulted in a cystic phenotype, indicating a functional link between induction of structural cystic abnormalities and the development of PKD. It seems that primary cilia act as mechano-sensors, since flow-induced bending leads to a rise in intracellular calcium levels. This appears to be modulated via the ciliary polycystin complex, with PC-2 mediating the flow-induced influx of extracellular calcium.

An interesting integrative hypothesis to explain how a defect in mechanosensation due to mutations of PC-1 or -2 may lead to cyst development...
was recently proposed by Lubarsky and Krasnow. Disruption of this pathway, caused for example by lack of PC-1 or -2, as in ADPKD, will lead to lack of apicobasal polarity with the appearance of apical markers, cell surfaces separate and pockets of fluid-filled lumen appear. These pockets of lumen coalesce and become continuous with the cyst lumen.

Apical membrane biogenesis also appears to be important in lumen formation. Epithelial secretion and apical membrane synthesis are thus processes required for tubule production. A major question is how do tubules know when to stop growing? When the tubular diameter reaches the required size, there must be a sensing mechanism which stops tubular growth, causing the cells to cease fluid secretion and proliferation and changing their phenotype to an adult, reabsorptive and non-proliferative phenotype. The loss of function of PKD1 or PKD2 in ADPKD results in excessive and uncontrolled tubular growth and fluid secretion; in other words, a loss of normal regulatory processes controlling tubule growth. Since ADPKD kidneys initially develop normally, PKD1 and PKD2 do not seem to be necessary for renal tubule formation, but may be required to regulate subsequent tubular growth. Thus these two genes may be part of a program regulating renal tubular size, by means of the mechanosensitive $Ca^{2+}$ signalling pathway in renal epithelial primary cilia that inhibits renal tubular growth. Primary cilia are activated by shear stress, which may be caused by expansion of the apical cell surface; PC-1 and -2 in the cilia will then activate the $Ca^{2+}$ signalling pathway and downstream events, inhibiting further expansion. Disruption of this pathway, caused for example by lack of PC-1 or -2, as in ADPKD, will lead to lack of inhibition of growth and continuation of unabated tubular expansion and secretion, as occurs in ADPKD. This could also explain why similar disorders of growth are also seen in ADPKD in other organs, such as hepatic and pancreatic cysts and vascular aneurysms.

A perplexing question regarding ADPKD is the nature of the mutational mechanism. Since the disease is autosomal dominant, all cells contain one normal gene and one mutated gene. Thus all cells should be able to produce normal PC-1 and -2. To explain this anomaly, the ‘two-hit’ hypothesis has been proposed. This suggests that in addition to the germ line mutation of one allele, a somatic mutation occurs in some cells of the second, normal allele, which thus becomes inactivated and unable to produce PC-1 or -2. Somatic mutations have indeed been found in cells from hepatic and renal cysts, supporting this hypothesis. It would neatly explain the focal nature of the disease and the striking phenotypic variation seen within families. A new study strongly supports the two-hit hypothesis: PC-1 and -2 normally cause an increase in intracellular calcium in response to shear stress, and it was previously reported that PKD1null/null mouse kidney epithelial cells (i.e. cells not expressing PC-1) were unresponsive to flow stimulation. In this study, PKD1+/null cells (i.e. heterozygous, and thus expressing PC-1) responded to shear stress by increasing intracellular calcium, similarly to normal cells. Cultured renal tubal cells from normal and non-dilated ADPKD human kidney tubules displayed normal ciliary expression of polycystins and responded to shear stress with the normal increase in intracellular calcium. In contrast, cultured cyst-lining epithelial cells from ADPKD patients with mutations in PKD1 or abnormal ciliary expression of PC-1 or -2 were unresponsive to shear stress. These data strongly support a two-hit hypothesis as a mechanism of cystogenesis.

Prospects for treatment

There is at present no specific treatment for ADPKD; treatment consists of the standard therapies for chronic renal disease, including good blood pressure control and control of hyperlipidaemia. A major problem in the evaluation of effectiveness of therapeutic interventions in ADPKD is that this is a very slowly evolving condition, and GFR is well maintained until relatively late in the course of the disease. Thus, use of GFR alone as a marker of disease progression over relatively short time periods is problematic, and ideally would require following patients for many years, which is generally not feasible. A critical requirement in interventional studies in human subjects is thus to identify surrogate markers of disease progression. A better indicator of disease progression than GFR would be successive evaluation of cyst size. The classic study of Dalgaard demonstrated a close connection...
between cyst size and loss of renal function in ADPKD. Thus a major potential aim of treatment would be to slow or prevent growth of cysts. A study has therefore been initiated by the NIH in order to test whether renal cyst size can be accurately assessed by CT or MRI, and whether these changes can be detected over relatively short periods of time.\(^{40,41}\) The first results of this study, involving 232 ADPKD patients studied by MRI over a period of three years, have just been published.\(^{42}\) Total cyst volume and total kidney volume increased exponentially, and a baseline total kidney volume >1500 ml was associated with a declining GFR. Thus kidney enlargement was continuous and quantifiable by MRI, and higher rates of enlargement were associated with more rapid decreases in renal function.

### Hypertensive control

The control of hypertension is the basis of any therapeutic regimen for chronic renal disease. Inhibition of angiotensin II, whether by ACE inhibitors (ACEI) or angiotensin receptor blockers (ARB) is effective in diabetic nephropathy and other chronic glomerulopathies.\(^{43}\) A recent meta-analysis studied eight randomized controlled trials including a total of 142 subjects with ADPKD.\(^{44}\) 48% of whom were randomized to ACEI and 52% to control. During a mean follow-up of 2.3 years, there was a significant decrease in protein excretion in the ACEI group, but no significant difference in disease progression as measured by change in serum creatinine. The relatively small number of patients and short period of follow-up did not allow any conclusions as to the effectiveness of ACEI in this population. A large, long-term study of effectiveness of ACEI on renal cyst growth in children and adolescents with ADPKD is currently under way.\(^{45}\)

An important point regarding antihypertensive treatment in ADPKD patients is the use of diuretics, which usually play an important role in the antihypertensive treatment of chronic renal disease. In ADPKD, there is a concentrating defect, and these patients are often polyuric.\(^{46,47}\) A similar concentration defect has been found in animal models of polycystic disease, and an upregulation of aquaporin 2 has been found, which is probably compensatory to the concentration defect.\(^{48-50}\)

It would seem \textit{a priori} counter-productive to use diuretics in these patients, because of the danger of inducing hypovolemia. Ecker \textit{et al.} compared two groups of patients with ADPKD, one receiving antihypertensive medications including diuretics, but without ACEI, and the other receiving ACE but no diuretics.\(^{51}\) The follow-up period was 5.2 years. A faster loss of function occurred in the diuretic group than in the ACEI group, and it would thus seem prudent to avoid use of diuretics in these patients.

### Use of statins

Dyslipidaemia is common in chronic renal disease, and is a risk factor for progression.\(^{52}\) HMG-CoA reductase inhibitors (statins) appear to be protective in several studies (summarized in reference 50). They also appear to have additional, ‘pleiotropic’ effects, including inhibition of proliferation of a number of kidney cell lines, induction of apoptosis, inhibition of chymokine and cytokine release by mesangial cells and monocytes, and inhibition of matrix components by mesangial cells. Cell proliferation is modulated via ras oncogenes, and ras production is increased in PKD. Farnesyl pyrophosphate, an intermediate in the conversion of acetyl Co-A to cholesterol, is required for the activation of ras-GTP-binding proteins, which are important in a number of cell functions, including cell proliferation. Statins reduce farnesyl production,\(^{53}\) and thus potentially ameliorate the accelerated cellular proliferation in PKD. Gile \textit{et al.}\(^{54}\) studied the effect of lovastatin in heterozygous (Cy+/) Han:SPRD rats from age 4–10 weeks, a period of rapid cystic disease progression in these animals. These rats are a model for ADPKD, but are somewhat different from the human form, having mainly proximal tubule cysts.\(^{55}\) In male rats, which are more seriously affected than females, lovastatin significantly reduced cystic kidney size, volume of cysts and levels of BUN. The short-term effects of statins in 10 normcholesterolaemic ADPKD patients were studied by Van Dijk \textit{et al.} in a double-blind cross-over study\(^{56}\) over a 4-week period. Simvastatin caused an increase in GFR, in effective renal plasma flow, and a significantly enhanced vasodilator response to acetylcholine in the forearm. These vascular effects could be due to an effect on endothelial function, and warrant further studies.

### C-myc antisense oligonucleotide treatment

The \textit{c-myc} oncogene is over-expressed in rodent PKD\(^{57,58}\) and in human ADPKD cells.\(^{59}\) This oncogene is associated with proliferation and apoptosis, which are characteristic of ADPKD, and appears to be involved in renal cystic disease, since \textit{c-myc} transgenic mice develop renal cysts,\(^{60}\) and revertant mice lose cysts.\(^{61}\) Antisense oligonucleotides can be used to inhibit expression of overexpressed mRNAs. An antisense oligonucleotide specific to \textit{c-myc} was used in
C57BL/6j-cpk mice, which have a rapidly developing renal cystic disease analogous to human autosomal recessive PKD, and inhibited progression of the disease.62 In the BALB/c-cpk cystic mouse, a model of autosomal recessive PKD with hepatobiliary pathology,63 both kidney and liver over-express c-myc mRNA. Treatment with antisense oligonucleotide ameliorated both kidney and biliary pathology.63 Human Phase 1b clinical trials are currently being performed by AVI Biopharma, evaluating pharmacokinetics and elimination of their c-myc antisense oligonucleotide in ADPKD patients [http://www.avobio.com/pr/pr160.html].64

EGFR tyrosine kinase inhibitors

The epithelial growth factor (EGF)-TGFα-EGF receptor (EGFR) axis plays an important role in promoting epithelial cell proliferation and cyst formation. Although EGF expression is decreased in animal polycystic kidney disease,65–68 EGFR expression is increased in cystic renal and hepatobiliary epithelia.69–71 EGFR tyrosine kinase inhibitors have been successful in inhibiting the disease process in bpk mice (a model of autosomal recessive PKD)72 and in the Cy rat (a model of ADPKD).73 However, neither of these models is entirely orthologous with human cystic disease. In Pck rats, a model of human ADPKD, EGFR tyrosine kinase inhibitors failed to affect the disease.74 At present, the role of EGF in ADPKD is unclear.

Sirolimus

In view of the importance of cellular proliferation in the pathogenesis of ADPKD, the powerful immunosuppressant and antiproliferative drug rapamycin (sirolimus) has been investigated in experimental PKD. Tao et al.75 showed that sirolimus markedly slowed disease progression in Han:SPRD (cy) rats, in terms of decreased proliferation in cystic and non-cystic tubules, inhibition of renal enlargement and cystogenesis, and prevented loss of renal function. Similar results were found by Wahl et al. in the same rat model.76 Sirolimus inhibits the protein kinase mTOR, which appears to be a major target of cell growth and proliferation. S6K is an effector protein downstream from mTOR, and total and phosphorylated levels of S6K were raised in heterozygous (cystic) animals. Sirolimus reduced both total and phosphorylated S6K. Further studies with sirolimus are in progress.76

Somatostatin

In a recent study, Ruggenenti et al. performed a randomized cross-over trial of somatostatin in 12 ADPKD patients over a period of 6 months.77 The rationale for this study was the clinical observation of prevention of disease progression over a period of 2 years in a woman receiving octreotide for other reasons. Progression of disease was measured as increase in kidney volume by CT. Somatostatin significantly slowed kidney volume increase. This interesting finding clearly requires a long-term study in a larger group of patients.

V2 receptor inhibition

The major advances in knowledge of the pathophysiology of cyst formation described above have indicated that cAMP plays a central role in the proliferation and secretion of cyst cells,2,13 and that cysts most commonly (if not always) arise from CCD principal cells.1,2 Thus inhibition of cAMP production would be a direct, physiological approach to reducing cyst expansion. Since vasopressin is the main stimulator of adenylyl cyclase, and thus of cAMP production in these cells, it is reasonable that blockade of vasopressin using vasopressin V2 receptor (VPV2R) antagonists could be therapeutic in polycystic disease. This has been tested using a non-peptide vasopressin antagonist (OPC-31260), which is 82 times more selective to rat V2 receptors than to rat V1a receptors.78 Torres et al. used the Pkd−/WS25 mouse, a mouse model of human ADPKD (PKD2) that reliably develops cysts after 3 months.50 OPC-31260 administered in the diet to these mice between 3 and 16 weeks of age markedly reduced renal cAMP production and inhibited disease development, as indicated by lower kidney weights, plasma BUN concentrations, renal cyst volumes, mitotic and apoptosis indices. The kidney weights of treated Pkd−/WS25 mice were similar to those of wild-type mice, indicating that renal enlargement had been prevented. Similar results were obtained in rat models of autosomal recessive PKD and nephronothisis.49 A derivative of OPC-31260 (OPC-41061, a more potent antagonist for human V2 receptors) is now being tested in phase II trials in ADPKD patients.

In the past few years, enormous advances in our understanding of the pathophysiology and genetics of this important and fascinating disease have suggested possibilities of new, effective treatments for reducing cyst growth and retarding disease progression. These trials are only beginning, but their results will be of great interest.

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References


