

# Polycystic kidney disease: will it become treatable?

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**Abstract:** Polycystic kidney disease is an inherited multisystem disorder. It causes progressive loss of kidney function, flank pain, urinary tract infection, arterial hypertension and vascular abnormalities. Until the present time the treatment of polycystic kidney disease has been symptomatic. New approaches based on cell culture of cyst wall epithelia and on the discovery of polycystins 1 and 2 have lead to novel treatment protocols to attack the origin of the disease. These protocols involve vasopressin antagonists, rapamycin and somatostatin at the present time.

**Key words:** polycystic kidney disease, rapamycin, vasopressin antagonists

Autosomal dominant polycystic kidney disease (ADPKD) is the commonest inherited progressive kidney disorder known. It affects several organs in addition to the kidneys such as the liver and the pancreas (cysts), the colon (diverticula), the heart (valvular defects), the aorta (aneurysms) and the cerebral arteries (aneurysms, subarachnoid hemorrhage). In affected patients several thousand cysts – although of microscopic dimensions – are usually present in each kidney at the time of birth. The cysts derive from collecting duct and proximal tubular epithelium. Slowly progressive enlargement of the renal cysts is usually associated with hypostenuria and nocturia in the teenage years and thereafter, with arterial hypertension in adulthood (25–30 years) and with progressive deterioration of kidney function after the age of 45 years. Urinary tract infection, renal stone disease and bleeding either into a cyst or into the urinary space all occur at an increased incidence; they cause fever, pain and colic. In exceptional cases of ADPKD the cystic disease of the liver is more severe than that of the kidney requiring liver transplantation before dialysis. Treatment of ADPKD used to be symptomatic; however there have been no proven treatments to prevent the progression of ADPKD towards stage 5 renal failure.

Over the last 15 years research into ADPKD has been propelled by a number of new discoveries:

- 1) it has become possible to study cyst lining cells in cell culture
- 2) there are now several animal models of cystogenesis in the rat and mouse
- 3) polycystins-1 and -2 have been described and the role of their mutations in causing ADPKD is beginning to be understood.

All this has lead to proposals for causative treatment strategies aiming to prevent the progression of cystic growth and renal failure in ADPKD. The following discussion is an attempt to outline major aspects of this research.

## Cell culture and the role of cyclic adenosine monophosphate

The analysis of cystogenesis by microdissection and cell culture of cystic tissue was first proposed by Wilson et al. in 1986 [1]. The authors obtained renal cysts from explanted kidneys of ADPKD-1 patients. They prepared cells and cell clusters from the cyst lining epithelia. These were seeded in cell culture – using defined culture media – and allowed to grow. Control experiments were conducted with epithelial cells obtained from normal human proximal tubulus and collecting ducts. The authors made 3 major observations:

- 1) the cystic derived epithelial cells grew out faster than the normal controls
- 2) in contrast to the confluent monolayer formed by normal control cells the cystic-derived monolayers formed lacunae – open spaces – spontaneously, termed “cyst-like areas”
- 3) the extracellular matrix and the basement membrane of the cystic-derived epithelia were abnormal. The observations thus suggested enhanced proliferation of cystic-derived cells, abnormal fluid-secretion into the “cyst like areas” and remodelling of extracellular matrix as peculiar features of renal cystic tissue in ADPKD-1; indeed these seminal observations have been confirmed in literally all subsequent research of ADPKD-1.

Grantham et al. [2] contributed to a principle understanding of renal cyst formation and enlargement. In their initial studies [2] they did not use ADPKD-1 derived cells but madin-darby canine kidney (MDCK) cells – a dog kidney derived tubular epithelial cell line showing distal tubular properties. The researchers discovered [2] that cell culture of MDCK cells in a collagen gel yielded MDCK cysts, i.e. a single layer of polarized cells enclosing a fluid – filled cavity. From an ob-

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served net increase of cystic volume over time together with a measured intracystic pressure that was higher than the pressure in the surrounding medium by 6.7 mm H<sub>2</sub>O the authors concluded that cysts grew as a consequence of net fluid secretion into the cysts. The direction of this transport was opposite to that of normal renal tubular epithelial cells that perform volume transport from apical to basolateral. In the model, inhibition of chloride transport slowed cyst expansion, implying that chloride transport was essential to cyst growth. In additional studies [3] the group of Grantham explored the factors controlling cystic enlargement. It was observed that agents which stimulated intracellular cyclic adenosine monophosphate (cAMP) such as arginine vasopressin, prostaglandin E1, forskolin and cholera toxin were pivotal and lead to MDCK cyst enlargement. Direct stimulation of intracellular cAMP with a phosphodiesterase inhibitor showed the same effects confirming the observations. The stimulation of cAMP appeared to be instrumental in two changes: increase of fluid transport into the cyst lumen; proliferation of cells in the cyst wall. The authors again noted a higher chloride concentration in the cyst fluid compared to the surrounding medium. This indicated that chloride transport was an important element for cyst enlargement. In additional studies the group of Grantham was successful in duplicating these observations in a model of human renal cortical epithelial cells [4] and in epithelium from human ADPKD-1 cysts [5]. Some years later when the genes of polycystins-1 and -2 and their mutations were described as the causes of ADPKD it was discovered that cAMP is antiproliferative in the presence of normal polycystin-1 but it turns pro-proliferative in (loss-of-function) mutations of polycystin-1 [6]. Recent animal studies have also supported a pivotal role of cAMP signalling in ADPKD. In these studies V2-receptor vasopressin antagonists – inhibiting the generation of cAMP – were effective inhibitors of cyst enlargement in the animals [7].

## Epidermal growth factor

Another pathway of stimulated proliferation of cystic tissue in ADPKD involves the epidermal growth factor (EGF) and its receptor. In 1995 Grantham et al. [5] had reported that cyst fluid harvested from a murine model of renal cystic disease caused MDCK cells in culture to proliferate, form cysts and secrete fluid into the cysts. The observed effects were comparable to those that had been found previously by the authors when they added EGF to the culture media [8]. Du and Wilson analyzed EGF and its receptor in cystic tissue from human ADPKD-1 *in vivo* and *in vitro* [9]. They showed high expression of EGF in cystic epithelia *in vivo* and they detected EGF in cyst fluid by radioimmunoassay. The levels were between 1.4 and 2.87 µg/ml and these concentrations had been shown previously to be highly mitogenic in ADPKD epithelia. The authors also analyzed ADPKD cystic tissue with respect to EGF receptors. They were able to delineate high – affinity binding sites in basolateral cell membranes – as occurred

in normal control epithelia – but they also found unique high affinity receptor binding in apical cell membranes of ADPKD cystic tissue, a feature that was absent in normal control epithelia [9]. The authors concluded that cyst expansion is mediated by an autocrine loop involving EGF synthesis by cyst epithelial cells, apical secretion into the cyst lumen and subsequent binding to abnormally located apical EGF receptors [9]. Since it became known later that the polycystins are membrane proteins contributing to cell polarity it is likely that mutations of polycystins cause an EGF receptor polarization defect to the apical membrane in ADPKD cyst epithelia. To test the pathogenetic role of autocrine EGF in renal cystic disease in a functional way, Torres et al. [10] treated an animal model of cystic disease – the Han: SPRD rat – with EKI-785, an inhibitor of EGF-receptor-tyrosine kinase. The EKI-785 in these studies was effective in lowering kidney weight, cyst volume and fibrosis scores and concentrations of blood urea nitrogen [10].

Taken together the EGF-EGF-receptor autocrine loop appears to provide a second powerful stimulus to proliferation of cells lining the cyst wall resulting in cyst enlargement. Attempts are being undertaken to utilize this concept in designing alternative therapies to retard the progression of ADPKD.

## The role of mammalian target of rapamycin

The activity of the kinase mammalian target of rapamycin (mTOR) in ADPKD has attracted attention [11]. It had been observed in animal models of polycystic kidney disease (PKD) that rapamycin slowed disease progression, and rapamycin is a known inhibitor of mTOR [12]. The mTOR has essential roles in protein translation, cell growth and proliferation and it is upregulated in several types of tumors [11]. Shillingford et al. [11] assessed the activity of mTOR by staining tissue sections for phospho-mTOR in human ADPKD tissue and in cysts of animal models for PKD. It was found that cystic epithelial cells – but not surrounding normal epithelium – expressed specific staining of phospho-mTOR [11]. Therefore the mTOR pathway is inappropriately activated in cystic epithelial cells in human ADPKD and in mouse models. In additional work Shillingford et al. [11] demonstrated that polycystin-1, mTOR and tuberlin form a multicomponent complex. The function of this protein complex is down-regulation of mTOR activity under normal conditions [11]. This would explain up-regulation of mTOR activity in the circumstance of a mutated polycystin-1. At the same time this line of evidence provides an explanation of the benefit from the mTOR inhibitor rapamycin in models of PKD [12]. Recently several trials in human ADPKD-1 testing rapamycin have been initiated.

## Apoptosis

Apoptosis and its possible role in progression of ADPKD-1 have intrigued researchers for some time [13]. In 1995 Woo

tested whether evidence of apoptosis could be found in kidneys from human ADPKD-1 [13]. He compared ADPKD-1 with renal tissue from IgA nephropathy, nephrosclerosis, diabetic nephropathy and acute renal failure in adequate numbers of specimens. Regardless of the presence or absence of renal failure it was found that only ADPKD-1 kidneys exhibited apoptotic cells and they were located in cyst walls, tubules and glomeruli [13]. The capacity of polycystic kidney cells to undergo apoptosis was retained *in vitro* in the absence of uremia and ischemia [13]. This work suggested for the first time that the functional deterioration of the kidneys in ADPKD-1 might be related to pathologic loss of renal tissue induced by abnormal apoptosis in addition to cell proliferation and progressive enlargement of renal cysts. The group of Edelstein used the inhibitor IDN-8050 of pro-apoptotic caspase-3 in the Han: SPRD rat model to examine the role of apoptosis in PKD in more detail [14]. IND-8050 significantly reduced the kidney enlargement by 44% and the cyst volume density by 29% in PKD rats [14]. This was associated with less of an increase of the blood urea nitrogen in the IND-8050 receiving PKD-rats but not in their untreated controls [14]. Interestingly, evidence has now shown that apoptosis under normal circumstances is controlled by polycystin-1. Boca et al. [15] found that the expression of full-length polycystin-1 in MDCK cells rendered them resistant to apoptosis. The implication is that a mutated – dysfunctional – polycystin-1 as occurs in ADPKD-1 may no longer be able to downregulate apoptosis. These findings open up an avenue of research in which the progression of human ADPKD-1 might be slowed by inhibitors of apoptosis – as has been shown in animal models [14].

As outlined in the previous text the pieces of the puzzle are beginning to come together. The discovery of polycystins-1 and -2 and their mutations has yielded an understanding of multiple changes that are in operation in ADPKD. These changes are: the pro-proliferative role of cAMP, the autocrine EGF loop, the activation of the kinase mTOR and the upregulation of apoptosis – at the present time. This work would have been impossible in the absence of suitable animal models, such as the pcy mouse, the pck rat, the cpk mouse and the Han: SPRD rat [16]. Based on the results of animal studies several clinical phase II/III trials have been initiated; they involve the V2-vasopressin antagonist tolvaptan and the insulin-like growth factor-1 antagonist somatostatin to prevent the build-up of cAMP; rapamycin to downregulate mTOR. Additional approaches, possibly targeting the autocrine EGF loop or apoptosis may become available in the near future. In all of these studies a yardstick shall be required to measure the benefit of the therapy. The glomerular filtration rate and the proteinuria will be unsuitable for this task because the clinical studies will be performed in stages 2 and perhaps 3 of renal failure, i.e. when functional changes are mild to moderate. It is therefore of interest that magnetic resonance tomography of total kidney volume and cyst size has

been suggested as a reliable and subtle marker of progression of ADPKD-1 [17] under these circumstances.

Taken together, the prospects for improved treatment(s) in polycystic kidney disease have never looked better than at present. A most impressive development of potential therapies from bench to bedside is fully underway. It is well justified for patients and physicians alike to look to the future of ADPKD-1 with major expectations.

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