Autosomal Dominant Polycystic Kidney Disease: Recent Advances in Pathogenesis and Treatment

Ming-Yang Chang\textsuperscript{a,b} Albert C.M. Ong\textsuperscript{a}

\textsuperscript{a}Academic Nephrology Unit, Sheffield Kidney Institute, School of Medicine and Biomedical Sciences, University of Sheffield, Sheffield, UK; \textsuperscript{b}Department of Nephrology, Kidney Research Centre, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan

\textbf{Key Words}
Autosomal dominant polycystic kidney disease \cdot Epithelial cyst \cdot Renal parenchyma \cdot Arterial aneurysms, intracranial

\textbf{Abstract}
Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder affecting 1 in 1,000 people in the general population and accounts for up to 10% of all patients on renal replacement therapy. Numerous fluid-filled epithelial cysts arise from different nephron segments as spherical dilatations or small out-pouchings, enlarge progressively and eventually become disconnected from the rest of the renal tubule. The development of cysts is accompanied by destruction of the renal parenchyma, interstitial fibrosis, cellular infiltration and loss of functional nephrons. ADPKD is not only a kidney disease but also a systemic disorder associated with intracranial arterial aneurysms, cardiac valvular defects, colonic diverticulosis and cyst formation in other organs such as the liver, spleen and pancreas. The identification of \textit{PKD1} and \textit{PKD2} together with the drive to elucidate the functions of their encoded proteins, polycystin-1 (PC1) and polycystin-2 (PC2), has led to an explosion of clinical and scientific interest in this common disorder. The aim of this review is to highlight recent advances in our understanding of ADPKD pathogenesis which are leading to exciting new treatment strategies.

\textbf{Molecular Genetics of ADPKD}

Autosomal dominant polycystic kidney disease (ADPKD) can arise from mutations in either \textit{PKD1} (85%) or \textit{PKD2} (15%) with clinically indistinguishable manifestations. However, PKD2 is usually of later onset since the mean age at end-stage renal disease (ESRD) occurs 16 years later than PKD1 (69.1 vs. 53.0 years) [1]. So far, no apparent clustering or ‘hot spot’ of mutations has been identified in either gene. Most mutations are predicted to truncate the protein or lead to its degradation. However, production of a partially functional mutant protein has not been excluded. The search for possible genotype/phenotype correlations has yielded mixed results. Mutations in the 5’/H11541 half of \textit{PKD1} (relative to the median nucleotide) have been shown to be associated with slightly earlier ESRD compared to mutations in the 3’ half of the gene and are also more strongly associated with aneurysm rupture [2, 3]. A clear genotype/phenotype correlation for PKD2 has not been found [4]. The considerable intrafamilial phenotypic variability, even in patients with identical mutations, reflects the strong influence of additional genetic modifiers and/or environmental factors on disease progression in ADPKD [5, 6].

Modifier genes could regulate the function of polycystin-1 (PC1) and polycystin-2 (PC2) by affecting polycystin-mediated signal transduction pathways, the rate of somatic mutations, cyst fluid accumulation or other clin-
ical factors associated with cyst initiation or disease progression [7]. In human ADPKD, these modifier genes are not well defined although several candidate loci have been investigated in small populations [8]. Confirmatory studies are required to clarify the modifying effects of these genes in larger well-defined ADPKD populations.

Mutational Mechanisms Underlying Cystogenesis

The mutational mechanism underlying cyst initiation in ADPKD remains controversial. Strong evidence for a two-hit mechanism has been presented which could help explain the focal nature of cyst development and the variability of the cystic phenotype [9, 10]. Nevertheless, there is accumulating data that haploinsufficiency itself may be sufficient to elicit a switch to a cystic phenotype. The strongest experimental evidence for this model comes from the generation of Pkd1 hypomorphs where a reduction, but not absence, of Pkd1 was sufficient to initiate renal and, in one study, extrarenal (vascular) features of ADPKD [11, 12]. Overexpression of PKD1 itself leads to cyst formation, implying that gene dosage is important [13, 14]. Of interest, Pkd1 heterozygous mice develop inappropriate anti-diuresis, and increased proliferative activity has been observed in the non-cystic tubules of Pkd2 heterozygous mice [15, 16]. Both Pkd1 heterozygous collecting ducts and Pkd2 heterozygous vascular smooth muscle cells have lower intracellular Ca$^{2+}$ levels [15, 17]. These findings appear to precede loss of a flow-sensitive (cilia) Ca$^{2+}$ response observed in PC1 null cells as the latter is retained in PKD1 or Pkd1 heterozygous tubular cells [18]. Thus, current evidence indicates that loss of a single allele of either PKD1 or PKD2 is sufficient to initiate phenotypic changes in tubular cells, activating a pathway which ultimately leads to cyst formation.

Functions of the Polycystin Proteins

**Polycystin-1 (PC1)**

PC1 is a 4,302-amino acid type I membrane glycoprotein containing a long N-terminal extracellular domain of over 3,000 amino acids, 11 transmembrane domains and a short cytoplasmic C-terminus (198 amino acids) [19]. PC1 interacts with PC2 via its C-terminus to form a heterodimeric complex. This primary interaction is critical for the functional regulation of both proteins [20].

A number of potential structural domains in the N-terminus may directly or indirectly (through E-cadherin or β1-integrin) mediate cell–cell or cell–matrix interactions [7]. A direct role of PC1 in mediating cell–cell adhesion via homophilic interactions of the PKD domains has been demonstrated [21]. Heterophilic interactions of other domains (LRR, C-lectin) with purified extracellular matrix proteins have also been described. Of interest, the entire N-terminal ectodomain has the biomechanical properties of a mechanosensor [22]. Potentially it could sensor laminar flow or the force of coupling between cells or cell–matrix attachments during tubulogenesis. These force-triggered events could in turn activate a PC2-dependent Ca$^{2+}$ signal and/or PC1-dependent signals. In addition to Ca$^{2+}$, the latter could include activation of monomeric GTPase proteins (Ras, Rho), heterotrimeric G proteins (Gi and Go), JAK/STAT, phosphatidylinositol 3-kinase (PI3-K)/Akt/mammalian target of rapamycin (mTOR) and activator protein 1 (AP-1)/mitogen-activated protein kinases (MAPK) pathways [7]. Finally, PC1 may undergo a stimulus-triggered proteolytic cleavage event to release its C-terminal tail – a process termed ‘regulated intramembrane proteolysis’ or RIP [23]. This fragment could translocate to the nucleus to directly initiate gene transcription.

**Polycystin-2 (PC2)**

PC2 is a 968-amino acid type II membrane glycoprotein with six transmembrane domains and intracellular N- and C-termini. PC2 (or TRPP2) has significant homology to the transient receptor potential (TRP) family of store-operated calcium channels and is likely to function similarly as a non-selective calcium channel [24]. Unlike Pkd1 mice, Pkd2 knockout mice were unexpectedly found to have a defect in left–right patterning of the body axis: PC2 (but not PC1) is expressed by monocilia at the mouse embryonic node and may mediate the asymmetric Ca$^{2+}$ signal proximal to the establishment of left–right asymmetry [25]. Similarly, PC2 (together with PC1) has been shown to mediate flow-induced Ca$^{2+}$ signals originating from primary cilia of kidney epithelial cells [26].

The subcellular location of PC2 has been an area of controversy with immunolocalisation to the endoplasmic reticulum (ER), lateral plasma membrane and primary cilia. PC2 does not contain a classical ER retention signal; rather, a stretch of acidic residues containing a casein kinase 2 phosphorylation site (Ser$^{812}$) appears to mediate its retrograde trafficking between the ER, Golgi and plasma membrane compartments [27]. A second phosphorylation site for PC2 within its N-terminal domain (Ser$^{26}$) is critical for its localization in the lateral plasma
membrane but not in the primary cilia [28]. A ciliary localisation signal in its N-terminus has also been described [29]. These three distinct non-overlapping recognition motifs could determine the subcellular location of PC2 and hence its function within different compartments. Finally, there is evidence that PC2 channel activity can be modulated by its interaction with the actin (lateral membrane?) or microtubule (cilia?) cytoskeleton [30, 31].

Mechanisms of Cyst Formation

Human and experimental data suggest several potential mechanisms that could lead to cyst formation in ADPKD: (1) increased cell proliferation and apoptosis; (2) enhanced fluid secretion; (3) abnormal cell–matrix interactions; (4) alterations in cell polarity, and (5) abnormal ciliary structure or function.

Cell Proliferation and Apoptosis

A primary increase in tubular proliferative activity, e.g. through the transgenic expression of oncogenes or growth factors, can lead to cyst formation [32]. PKD1 cystic epithelial cells are more sensitive to the mitogenic effect of growth factors and cAMP in vitro – these effects are dependent on Ras and Raf activity [33]. ADPKD cystic cells also have lower intracellular Ca<sup>2+</sup> concentrations and this appears to correlate with their responsiveness to cAMP via a B-Raf/ERK pathway [34]. A potential approach to inhibit cAMP-dependent cell proliferation could be by restoring intracellular Ca<sup>2+</sup> regulation in these cells – this may be the basis of action of triptolide, a recently described natural compound, on cyst formation in Pkd1 null mice [35].

A primary defect in apoptotic regulation (e.g. bcl-2 null mice) can result in a cystic phenotype [36]. In human ADPKD tissue, high levels of c-myc expression have been found [37]. Moreover, kidney-specific overexpression of c-myc (SBM mice) leads to a cystic phenotype and an increase in both proliferation and apoptosis [38]. Conversely, transgenic expression of human PC1 in MDCK cells can induce cell proliferation and apoptosis suggesting that, like c-myc, PC1 could exert independent effects on both cellular processes [39].

Fluid Secretion

In patients with ADPKD, the tubular epithelium appears to switch from an absorptive to a secretory phenotype. Tubular secretion is primarily mediated by trans-epithelial Cl⁻ secretion with passive Na<sup>+</sup> and water movement [40]. Chloride transport could be mediated by several channels including the cystic fibrosis transmembrane conductance regulator and Ca<sup>2+</sup>-sensitive Cl⁻ channels [41, 42]. Intracellular cAMP and extracellular ATP may be involved in regulating this process [43, 44]. Recently, forskolin, a potent stimulator of adenylyl cyclase, has been isolated from human ADPKD cyst fluid samples [45].

Cell–Matrix Interactions

A primary defect in laminin synthesis has recently been shown to cause PKD in a study describing a hypomorphic allele of laminin α₅ [46]. ADPKD cyst epithelia sit on an expanded basement membrane and abnormal increases in basement membrane components (laminin, fibronectin, type IV collagen, and heparan sulphate proteoglycan), and interstitial type I collagen have been reported. These changes in basement membrane composition could contribute to cyst initiation or expansion. PCI-deficient cystic cells have been shown to adhere more strongly to type I collagen by a β₁-integrin-dependent mechanism and may mediate resistance to anoikis [47, 48].

Cell Polarity

Alterations in apicobasal or planar cell polarity have been described in several cystic cell models, although not always consistently. Mislocation of E-cadherin (to the apical surface) could impair the subsequent targeting of protein assemblies crucial for basolateral trafficking and could account for the observation of mislocalised basolateral proteins (e.g. EGF receptor, Na<sup>+</sup>-K<sup>+</sup>-ATPase) in ADPKD cyst epithelia [49]. Alternatively, abnormalities in planar cell polarity (Wnt signalling in NPHP2/inv mice) or oriented cell division (spindle orientation in HNF-β1 mice) during tubular lengthening could be key mechanisms underlying cyst formation in some recessive models of PKD or nephronophthisis [50, 51].

Ciliary Structure and Function

A recent major theme in the elucidation of PKD pathogenesis is the association between structural abnormalities of the primary cilia (a previously neglected organelle) and a cystic phenotype [52]. Among several seminal observations were the findings that the worm pkd1 and pkd2 orthologues are functionally important in mechanosensory neuronal cilia and that PKD mice hypomorphic for Tg737/polaris have short kidney primary cilia [53, 54]. Many cystoproteins have since been immunolocalised to primary cilia or centrosomes (which give rise to primary cilia) and
Chang / Ong
Nephron Physiol 2008;108:p1–p7

... have been associated with structural or functional abnormalities of these organelles. Indeed, kidney-specific inactivation of Kif3A, a protein involved in the maintenance of renal ciliary structure, can itself lead to PKD [55].

In PKD1, the major ciliary abnormality does not appear to be structural, but rather functional: Pkd1 null collecting duct cells or human cyst-lining cells have defects in flow-induced ciliary Ca$^{2+}$ signals [18, 26, 56]. How abnormalities in ciliary Ca$^{2+}$ signals lead to the complex cystic phenotype of ADPKD cells (see above) and hence cystogenesis itself is the focus of intense investigation.

### Treatment Targets for ADPKD

Figure 1 summarises some of the major advances in our present understanding of the cellular mechanisms underlying the cystogenesis in ADPKD. These in turn have led to some interesting new molecular targets as potential treatments (table 1). Overall, these approaches can be summarised into two major ones: targeting cell proliferation and lowering cAMP.

In view of the pronounced increase in tubular proliferation, several groups have attempted an anti-prolifera-
tive strategy in experimental models. Examples of these are the use of rapamycin (to inhibit mTOR), R-roscovitine, orpk and bpk mouse models [57]. Treatment with the cyclin-dependent kinase inhibitor R-roscovitine effectively arrested cystic disease in jck and cpk mice [58]. Administration of EGFR receptor tyrosine kinase inhibitors, EKB-569 and EKI-785, markedly reduced cyst formation in bpk mice and Han:SPRD rats, though not in the PCK rat. Alternative approaches to block downstream mitogenic signalling intermediates (e.g. ERK) have also been attempted [59].

In view of its stimulatory effects on cell proliferation and fluid secretion, a second major approach has been to target renal cAMP production. In landmark studies, administration of the vasopressin V2 receptor (V2R) antagonist, OPC31260, effectively inhibited disease development and progression in three rodent models – pcy mice, PCK rats and Pkd2/– mice [60]. Vasopressin antagonism was associated with a decrease in kidney cAMP concentrations. Similarly, somatostatin treatment (which can inhibit cAMP-stimulated chloride secretion) led to a slowing in the rate of cyst volume expansion in a small study of ADPKD patients and in PCK rats [61]. The role of vasopressin action in modifying cyst expansion in ADPKD has been given added weight by recent studies which indirectly suppressed (high fluid intake) [62] or enhanced its action (endothelin B receptor blockade) [63] on the collecting duct.

Table 1 summarises other potential approaches that have been attempted in PKD models. Other features associated with cyst enlargement such as apoptosis, inflammation or interstitial fibrosis could represent alternative treatment targets. One note of caution is necessary when directly extrapolating results from cystic rodent models for human ADPKD. Clearly not all cystic diseases are the same. Apart from obvious differences in genotype, cysts may originate from different nephron segments and this could influence the effectiveness of different drugs [64]. We suggest that preclinical testing of candidate drugs should include the use of orthologous models of ADPKD as a minimum.

At present, blood pressure control remains the major treatment modality though currently, at least 3 major clinical trials seeking to alter the natural history of the disease (tolvaptan, rapamycin and somatostatin) are in progress. Given the complexity of the cystic disease process, it seems likely that a combination strategy will be required for maximal therapeutic benefit. The next decade holds much promise of an effective treatment for a once untreatable disease.

**Acknowledgements**

Work in the author’s laboratory was supported by the Wellcome Trust, Polycystic Kidney Disease Foundation (USA), Research Councils UK, Kidney Research UK and the Medical Research Council. We apologise for omitting a more comprehensive bibliography due to space limitations.

**References**


46 Shannon MB, Patton BL, Harvey SJ, Miner PD, Gusella GL: Stable knockdown of poly-
cystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. Proc
Natl Acad Sci USA 2003;100:5286–5291.
47 Wilson PD, Geng L, Li X, Burrow CR: The PKD2 gene product, ‘polycystin-1,’ is a tyro-
sine-phosphorylated protein that colocalizes with alpha2beta1-integrin in foci in clusters in
48 Battini L, Fedorova E, Macip S, Li X, Wilson PD, Gusella GL: Stable knockdown of polycy-
2006;17:3049–3058.
49 Charron AJ, Nakamura S, Bacallao R, Wanding-Ness A: Compromised cytoarchitec-
ture and polarized trafficking in autosomal dominant polycystic kidney disease cells. J Cell
50 Simons M, Gloy J, Ganner A, Bullerkott A, Bashkurov M, Kronig C, Schermer B, Ben-
zing T, Cabello OA, Jenny A, Mlodzik M, Bashkurov M, Kronig C, Schermer B, Ben-
zing T, Cabello OA, Jenny A, Mlodzik M, Metz T, Doyen A, Nato F, Nicolas JF, Torres V, Yanz M, Pontoglio M: Defec-
51 Fischer E, Legue E, Doyen A, Nato F, Nicolas JF, Torres V, Yanz M, Pontoglio M: Defec-
52 Ong AC, Wheatley DN: Polycystic kidney disease – the ciliary connection. Lancet
53 Barr MM, Sternberg PW: A polycystic kidney disease gene homologue required for
54 Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL: Dof1, a C. elegans homolog of
PKD1 gene product, ‘polycystin-1,’ is a tyrosine kinase inhibitor. Proc Natl Acad Sci USA
55 Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somolo S, Igarashi P: Kidney-
specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and
epithelial cells with a novel, single codon dele-
elion in the PKD1 gene exhibit defective cili-
ary polycystin localization and loss of flow-
57 Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, Flask CA,
Novick AC, Goldfarb DA, Kramer-Zucker A, Walsh G, Piontek KR, Germino GG, Weimbs T: The mTOR pathway is regulated by poly-
cystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. Proc
Natl Acad Sci USA 2006;103:5466–5471.
58 Bukanov NO, Smith LA, Klinger KW, Led-
nermorel N, Morente N, Kranzlin B, Gretz
59 Muto S, Aiba A, Saito Y, Nakamura K, Nakamu-
ra K, Tomita K, Kitamura T, Kurabayashi M,
Nagai R, Higashihara E, Harris PC, Katsuki M, Horie S: Pioglitazone improves the pheno-
type and molecular defects of a targeted Pkd1 mutant. Hum Mol Genet 2002;11:1731–
1742.
60 Torres VE, Sweeney WE, Jr., Wang X, Qian Q, Harris PC, Frost P, Avner ED: Treatment of polycystic kidney
disease with a novel tyrosine kinase inhibi-
stein FH: Safety and efficacy of long-acting somatostatin treatment in autosomal-domi-
62 Nagao S, Nishii K, Katsuyama M, Kurahashi H, Marunouchi T, Takahashi H, Wallace DP: Increased water intake decreases progres-
63 Chang MY, Parker E, El Nahas M, Haylkr J, Ong AC. Endothelin B receptor blockade ac-
celerates disease progression in a murine model of autosomal dominant polycystic kidney
64 Guay-Woodford LM: Murine models of polycystic kidney disease: molecular and therapeu-
65 Tao Y, Kim J, Schrier RW, Edelstein CL: Ra-
pamycin markedly slows disease progression in a rat model of polycystic kidney disease. J
66 Sweeney WE, Chen Y, Nakanishi K, Frost P, Avner ED: Treatment of polycystic kidney
disease with a novel tyrosine kinase inhibi-
67 Torres VE, Sweeney WE, Jr., Wang X, Qian Q, Harris PC, Frost P, Avner ED: EGF recep-
tor tyrosine kinase inhibition attenuates the development of PKD in a mouse model of ADPKD. J
68 Obermuller N, Morente N, Kranzlin B, Gretz
N, Witzgall R: A possible role for metallopro-
69 Faubel S, Wu JC, Falk SA, Schrier RW, Edelstein CL: Caspase inhibition reduces tubular apoptosis and prolifera-
tion and slows disease progression in poly-
Muto S, Aiba A, Saito Y, Nakao K, Nakamura
zene improves the pheno-
type and molecular defects of a targeted Pkd1 mutant. Hum Mol Genet 2002;11:1731–
1742.