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Review

The polycystins: a novel class of membrane-associated proteins involved in renal cystic disease

R. Sandford[a,*], S. Mulroy[b] and L. Foggensteiner[b]

[a]Department of Medical Genetics, Level 4, Cambridge Institute of Medical Research, Wellcome Trust/MRC Building, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2XY (UK), Fax + 44 1223 331206, e-mail: rns13@cam.ac.uk
[b]Department of Medicine, Cambridge Institute of Medical Research, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2XY (UK)

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Abstract. Polycystin-1, polycystin-2 and polycystin-L are the predicted protein products of the PKD1, PKD2 and PKDL genes, respectively. Mutations in PKD1 and PKD2 are responsible for almost all cases of autosomal dominant polycystic kidney disease (ADPKD). This condition is one of the commonest mendelian disorders of man with a prevalence of 1:800 and is responsible for nearly 10% of cases of end-stage renal failure in adults. The cloning of PKD1 and PKD2 in recent years has provided the initial steps in defining the mechanisms underlying renal cyst formation in this condition, with the aim of defining pharmacological and genetic interventions that may ameliorate the diverse and often serious clinical manifestations of this disease. The PKD genes share regions of sequence similarity, and all predictintegral membrane proteins. Whilst the predicted protein domain structure of polycystin-1 suggests it is involved in cell-cell or cell-matrix interactions, the similarity of polycystin-2 and polycystin-L to the pore-forming domains of some cation channels suggests that they all form subunits of a large plasma membrane ion channel. In the few years since the cloning of the PKD genes, a consensus that defines the range of mutations, expression pattern, interactions and functional domains of these genes and their protein products is emerging. This review will therefore attempt to summarise these data and provide an insight in to the key areas in which polycystin research is unravelling the mechanisms involved in renal cyst formation.

Key words. Polycystic kidney disease; polycystin; mutations; renal cysts; antibodies; immunolocalisation; signal transduction.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) has been the subject of considerable clinical and scientific interest over many decades. It is a common genetic condition that is inherited as an autosomal dominant trait and affects in excess of 1:1000 of the general population, which represents more than 5 million people worldwide [1]. It is a systemic condition with diverse clinical abnormalities, cystic and noncystic, seen in renal and extrarenal tissues [2]. Disease expression is extremely variable, even within families, which strongly suggests that other genetic or environmental factors may have a role in cyst formation and disease progression [3, 4]. The mechanisms underlying renal cyst formation in ADPKD and other inherited and

* Corresponding author.

acquired conditions are largely unknown. The opportunity to identify the processes involved in cyst formation in this common monogenic condition may therefore provide valuable insights into normal renal cell physiology and the function of other tissues affected in ADPKD, especially the cardiovascular system. The elucidation of the function of the ADPKD genes and the identification of other disease-modifying factors also provides the best opportunity to design interventions that may significantly alter disease progression.

A wealth of data has been collected from a varied multidisciplinary approach to the study of ADPKD which has defined the common anatomical, biochemical and physiological abnormalities seen in renal cysts [5, 6]. Polycystic kidney diseases have been defined as 'genetic or acquired disorders with progressive distension of multiple tubular segments or glomerular capsules, and are manifested by fluid accumulation, growth of non-neoplastic epithelial cells and remodelling of the extracellular matrix resulting ultimately in some degree of renal functional impairment, with the potential for regression after removal of the inductive agents' [7]. Cysts arise from the epithelial cells lining the renal tubule and may occur due to a wide variety of experimental and disease processes, genetic and nongenetic [8–10]. Cysts are seen in the normal ageing population, in the kidneys of individuals with endstage renal failure (ESRF) whose primary diagnosis is a noncystic disorder, and most commonly in the genetic polycystic diseases, ADPKD and autosomal recessive polycystic kidney disease (ARPKD). In ADPKD cysts arise in only a small percentage of nephrons, suggesting that a single PKD mutation is not sufficient by itself to initiate cyst formation. Whilst environmental influences may be involved, it has been suggested that two genetic hits may be required for cyst formation: an inherited germline mutation and an acquired somatic mutation [11]. Focal cyst formation in ADPKD may therefore be analogous to tumour formation in genetic cancer syndromes.

Cyst formation represents one of the limited responses to injury that the kidney may undergo and is likely to be the visible consequence of abnormalities that affect a common final set of cellular functions in renal tubular epithelial cells. As ADPKD is a single gene disorder, studies in this condition will form an important part in dissecting the disease-specific and more general pathways involved in normal renal epithelial cell function and cyst formation. Common abnormalities exist in renal cystic diseases, including abnormal epithelial cell proliferation, abnormal extracellular matrix production and degradation, and altered fluid secretion and cell polarity. It has been suggested that many of these changes may be explained by an alteration in the state of differentiation of the tubular epithelial cells [12]. The protein products of the PKD genes, the polycystins, may therefore be involved in pathways involved in initiating and maintaining tubular cell differentiation.

There is an increasing awareness of the systemic nature of ADPKD. Whilst the renal cystic disease predominates in most ADPKD families, many extrarenal features of the disease, both cystic and noncystic, are clinically important. The renal disease in ADPKD comprises progressive renal cyst formation and enlargement with the development of renal insufficiency in the majority of affected individuals [13]. This often progresses to ESRF, with $\sim 50\%$ requiring renal replacement therapy (dialysis or transplantation) during their 6th decade. Patients with ADPKD account for approximately 10% of adult cases of ESRF. Other renal complications include massive renal enlargement, cyst haemorrhage and infection, and renal stone formation. Rarely, the cystic manifestations may present in young infants or be detected in utero [14]. This form of ADPKD is often associated with a severe clinical course in infancy, with hypertension and ESRF occurring in childhood. Parents who have had one pregnancy or infant affected with this severe form of ADPKD are at high risk of having a further child with similar features. This risk is not evident in second degree relatives who also have the same PKD mutation, strongly suggesting that coinheritance of a modifying gene from the unaffected parent is responsible for altering disease expression [3].

The most common extrarenal manifestation of ADPKD is hepatic cysts [15]. This is rarely associated with impairment of hepatic function but may present with severe discomfort and abdominal distension in a small number of individuals, usually females. Complications such as cyst haemorrhage and infection may be life-threatening [16]. Cysts may also be found in the pancreas. Of the noncystic manifestations, those in the cardiovascular system predominate. Hypertension is a frequent feature of ADPKD, occurring in up to 75% of patients often well before detectable renal impairment is apparent [17, 18]. Cardiac valvular abnormalities are also commonly reported, with mitral valve prolapse occurring in 25% of patients. Left ventricular hypertrophy, aortic root dilation and congenital heart disease are also seen and contribute to the broadening spectrum of cardiovascular disease seen in ADPKD [19–21]. Intracranial aneurysms (ICAs) occur in 8% of ADPKD patients compared with $\sim 1\%$ in the non-ADPKD population and may represent a further focal manifestation of the disease [22]. Rupture leading to subarachnoid haemorrhage occurs at a younger age and has a more severe outcome compared with non-ADPKD ICAs, although the precise natural history of this condition in ADPKD is still unclear. More generalised connective tissue abnormalities have been described in ADPKD,

with colonic diverticular disease and abdominal wall herniae seen more commonly than in the non-ADPKD population [23, 24]. The major causes of premature morbidity and mortality in ADPKD following the introduction of renal dialysis in the 1970s are cardiovascular disease and sepsis [25].

Abnormalities in ADPKD are both focal, cyst and aneurysm formation, and generalised, hypertension and connective tissue features. Experimental studies examining PKD gene expression and the mutational mechanisms underlying cyst formation are now starting to elucidate the pathological mechanisms for these clinical observations.

The PKD gene family

Mutations in two genes have been shown to be responsible for virtually all cases of ADPKD [26]. These genes, PKD1 and PKD2, share some sequence similarity, and both predict integral membrane proteins [27, 28]. They differ considerably in their predicted domain structure and genomic organisation. A further gene, PKDL, which has high homology to PKD2 but which has not been implicated in human polycystic kidney diseases, was recently described [29].

The PKD1 gene

PKD1, the major ADPKD locus, maps to the telomeric end of the short arm of chromosome 16 (16p13.3) [30]. A chromosome translocation that segregated with the disease in a single Portuguese family eventually identified the PKD1 gene after a decade pursuing the gene by standard positional cloning strategies [31]. It was found to be adjacent to the TSC2 gene in a genomic region that is duplicated several times more proximally at 16p13.1. The 14-kb PKD1 gene was shown to be widely expressed in tissues and cell lines by Northern analysis and RNAase protection assays and to be conserved in other mammalian species [31]. Complete characterisation of the PKD1 gene was finally achieved using genomic sequencing and a novel exon-linking strategy [23, 32, 33].

The PKD1 gene spans over 50 kb of genomic DNA and consists of 46 exons. The 14.1-kb transcript predicts a 4302-amino acid membrane-spanning glycoprotein, polycystin-1, of molecular mass ~ 460 kDa [28]. The presence of multiple transmembrane regions identifies a large extracellular portion consisting of multiple structural motifs (fig. 1). The extracellular region of ~ 3000 amino acids includes a signal peptide, two leucine-rich repeats (LRRs), a C-type lectin domain, 16 PKD repeats and an REJ (receptor for egg jelly) module [28, 32, 34]. This domain structure is conserved across vertebrate evolution in the *Fugu* PKD1 gene [35]. Minimal sequence similarity between the human and Fugu genes suggests that it is the structural properties of each domain that are important rather than conserved sequence.

The most N-terminal domain is the LRR. Two repeats are found flanked on either side by cysteine-rich regions. The LRR is a short consensus sequence, often present in tandem, usually of 24 residues with precisely spaced leucines, that is present in many proteins of diverse function involved in protein-protein interactions [36]. Many of these interactions form part of signal transduction pathways, and the presence of the flanking regions is usually associated with adhesive proteins and receptors. The C-type lectin domain may also be involved in cell adhesion. This extracellular domain is found in a diverse range of proteins where it binds carbohydrate in the presence of Ca^{2+} [37]. The N-terminal region of polycystin-1 therefore contains protein motifs that predict a role in cell adhesion.

The LRR is separated from the C-type lectin domain by a single PKD domain. A further 15 PKD domains are arranged in tandem. In total these domains, which were noted to be similar to immunoglobulin (Ig) domains, comprise $\sim 30\%$ of the whole protein [28]. The structure of this domain has now been determined [38]. The PKD domain is a novel protein domain with a β sandwich or Ig fold (fig. 2). They are not, however, members of the Ig superfamily. Two β sheets, which are packed face to face, surround a well-defined hydrophobic core. The WDFGDGS motif, which is the most conserved sequence between the PKD domains, forms part of this core. The function of the PKD domain is not known nor whether they pack together and form a spacer region between the plasma membrane and the adhesive N-terminus or are involved in interactions with other extracellular proteins. Potential binding sites include both strands and loops, and the identification of pathogenic missense mutations in this region will help to determine the role of these domains in polycystin function [38].

In the original model of polycystin-1 proposed by Hughes et al., four fibronectin type III domains were located between the PKD domains and the first transmembrane domain [28]. This region has now been found to have similarity to the sea urchin sperm protein REJ [34]. This protein interacts with surface proteins in the egg jelly during fertilisation to induce the ion channel-regulated sperm acrosome reaction. A human orthologue may also have the same function [39]. The whole extracellular region of polycystin-1 is therefore comprised of domains that may be involved in protein-protein interactions, suggesting that many different extracellular ligands may be involved in polycystin function.

Comparative analysis with the Fugu PKD1 gene has also redefined the likely membrane topology of polycystin-1 [35]. Eleven transmembrane domains can be predicted with the first one corresponding to the REJ single transmembrane domain and 6–11 corresponding to the six domains in polycystin-2. Overall homology between the human and Fugu genes is low, which readily highlights areas of similarity. The most highly conserved region of PKD1 is located in the first intracellular loop. This suggests an important functional role that remains to be determined. A conserved coiled-coil domain is present in the C-terminal region that is involved in polycystin-1/polycystin-2 interactions.

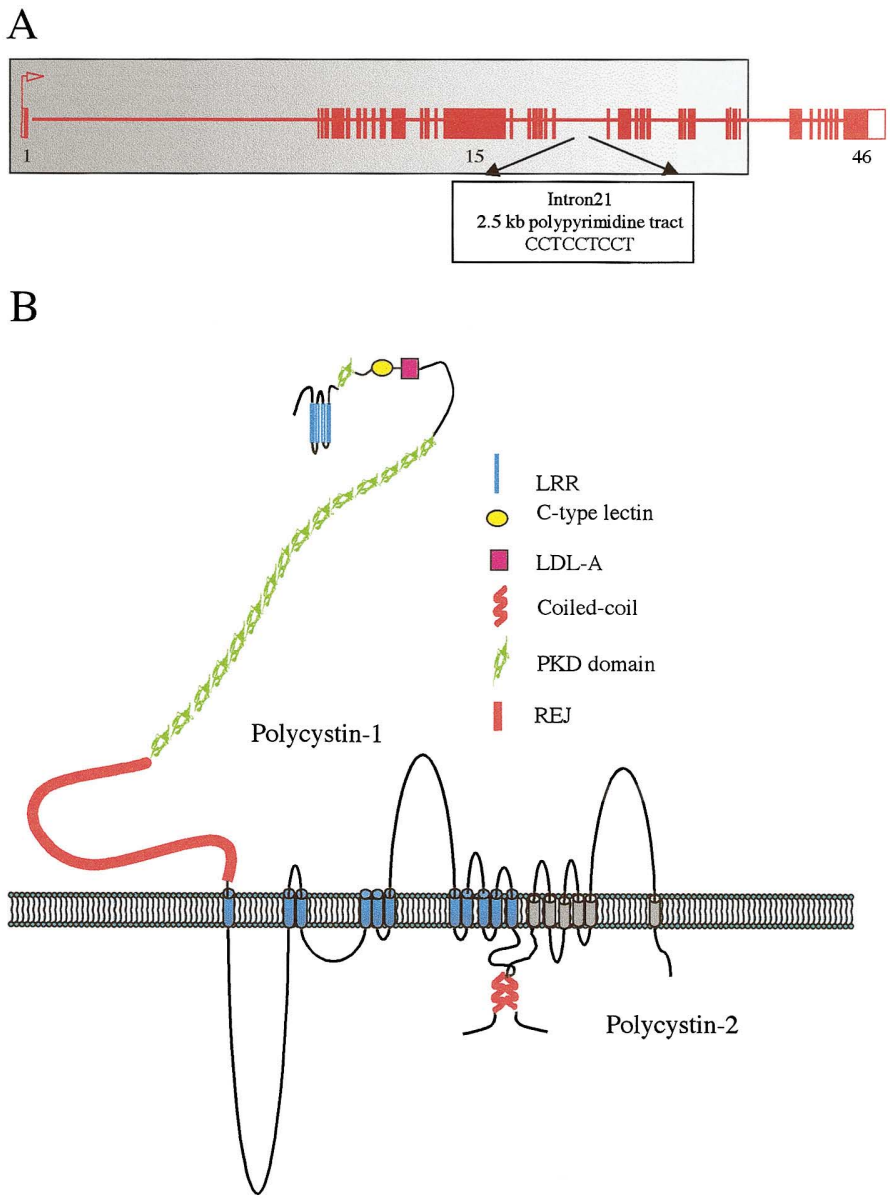

Figure 1. The PKD1 gene and its predicted protein product, polycystin-1. (*A*) The PKD1 gene contains 46 exons. The majority of the 5′ region of the gene (grey shaded area) up to exon 34 is duplicated several times also on chromosome 16. Intron 21 contains a large polypyrimidine tract that may contribute to the high new mutation rate seen in the PKD1 gene. (*B*) The predicted structure of polycystin-1 included multiple extracellular domains, a transmembrane region of up to 11 domains and a cytoplasmic tail. A coiled-coil domain in the cytoplasmic region of polycystin-1 interacts with a predicted coiled-coil domain in polycystin-2.

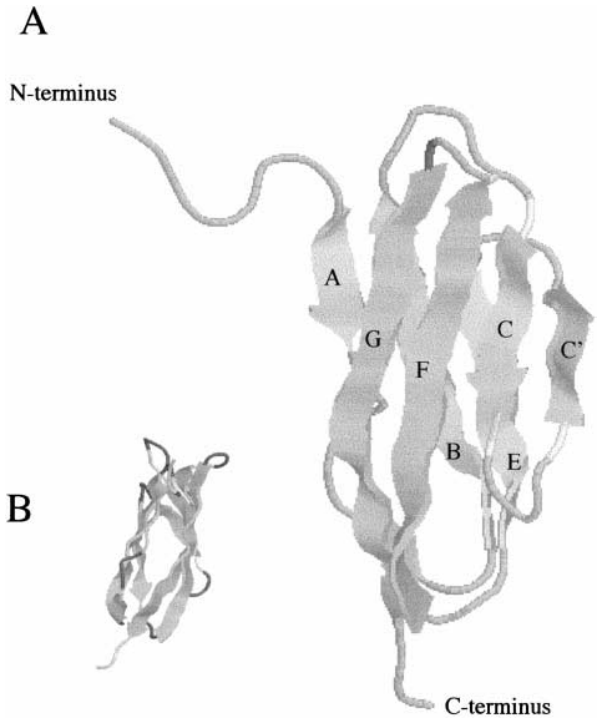

Figure 2. The PKD domain of polycystin-1. This domain is made up of a characteristic β sandwich or Ig fold. (*A*) One β sheet contains three strands, A, B and E, with the other sheet containing four, G, F, C and C′. (*B*) The two sheets are arranged in a 'sandwich' around a conserved hydrophobic core.

PKD1 therefore encodes the first member of a novel protein family that is made up of a unique array of protein structural domains that may be involved in cell-cell or cell-matrix interactions.

The PKD2 gene

Mutations in the PKD2 locus at 4q21–23 account for ~15% of cases of ADPKD. The PKD2 gene was identified in 1996 by positional cloning, although its presence in the expressed sequence tag (EST) databases had been detected because of sequence similarity to PKD1 [27, 40]. The 5-kb transcript consists of 15 exons spanning ~68 kb of genomic DNA and is single copy [41]. The predicted protein, polycystin-2, is a 968-amino acid protein with six potential membrane-spanning regions and intracellular N- and C- termini [27]. Clues to its potential function come from its sequence similarity to polycystin-1 and the α1 subunits of a family of voltage-activated Ca^{2+} channels. This homology extends between membrane spans 2–6 with the Ca^{2+} channels and includes the pore-forming region. The similarity with polycystin-1 extends further, with all six transmembrane domains of polycystin-2 aligning with the last six of polycystin-1 [35]. The C-terminal portion of polycystin-2 also contains regions of potential functional significance. In addition to a predicted coiled-coil domain, an EF-hand domain is present [27, 42]. This specialised helix-loop-helix motif may play a role in Ca^{2+} binding and regulation of ion channel function. With the possible function of the polycystin-1 REJ domain in ion channel-regulated processes, these predictions for polycystin-2 function have a consistent theme. Identification of further sequences from the EST databases with homology to PKD2 has resulted in the recent identification of PKDL, which encodes a new member of the polycystin protein family [29]. It has over 70% amino acid similarity with polycystin-2 in addition to similarity with pore-forming subunits of Ca^{2+} channels. Similarity to polycystin-2 extends to the prediction of six possible membrane-spanning regions, the presence of an EF-hand domain and a coiled-coil domain in the C-terminal region. A polycystin motif consisting of three to four basic residues is found in the first extracellular loop of polycystin-2 and polycystin-L. Its presence in polycystin-1 suggests functional significance. Polycystin-L may therefore interact directly with the other polycystins to form a large multimeric membrane-spanning ion channel. Regulation or gating of this channel and the control of cellular Ca^{2+} ion concentrations may be achieved by the interaction of polycystin-1 with its extracellular ligands. Whilst PKDL is not implicated in ADPKD at present, it has been found to be deleted in Krd (kidney and retinal defects) mice, which exhibit a range of renal phenotypes including cystic dysplasia.

One hit or two mutations in PKD1 and PKD2

Numerous mutations identified in PKD1 and PKD2 have confirmed their role in ADPKD [43–49]. The majority of mutations in PKD2 are nonsense mutations and result in protein truncation, suggesting that gene inactivation is the main mutational mechanism in PKD2 [45, 50]. Mutation analysis in PKD1 has been hampered by the presence of multiple homologous copies elsewhere on chromosome 16 [31]. Only the 3′ end of the gene is single copy, and most PKD1 mutations described are in this region (fig. 1). Most mutations are private, and no clustering within the PKD1 gene has been found to date. The majority predict a prematurely truncated protein and are likely to be inactivating as seen in PKD2 mutations [47]. Until the rest of the gene can be completely analysed, the full range of PKD1 mutations and the contribution of missense changes to the development of ADPKD remains to be determined. A relatively high new mutation rate of ~10% has been observed in ADPKD [51]. Several aspects of PKD1 gene structure may contribute to this.

The presence of a large polypyrimidine tract in intron 21 that has the potential to form triple helix structures may predispose to a high mutation rate in this region [33, 49]. Clustered base changes in exon 23 have been shown to be due to gene conversion with the homologous genes [52]. These highly homologous copies may therefore act as a reservoir of mutations for the duplicated region of the gene. Only analysis of the complete PKD1 gene will determine whether mutations cluster around exons 20 and 21 and how many can also be identified in the homologous genes. The development of long-range PCR and reverse transcriptase PCR (RT-PCR) strategies that are PKD1-specific will allow the full range of PKD1 mutations to be determined [49, 53].

The focal nature of ADPKD has suggested that other genetic or environmental effects are required for cystogenesis in addition to a PKD1 or PKD2 mutation. Convincing evidence for a second genetic hit has come from the demonstration of loss-of-heterozygosity and somatic PKD1 mutations in epithelial cells isolated from renal and hepatic cysts [54–57]. In addition to the inherited germline PKD1 mutation, renal and hepatic cyst lining cells have been shown to have acquired somatic mutations in the normal allele. This 'two-hit' mechanism is analogous to the two-hit model of tumour formation proposed by Knudson which has been demonstrated in many familial cancer syndromes and other nonmalignant conditions characterised by focal benign tumour formation [58]. Random second hits may also occur in other genes that regulate renal epithelial cell function, so the two-hit hypothesis may provide an explanation for the focal nature of the disease and also for the marked clinical variability seen within the same ADPKD family. The late onset appearance of renal and hepatic cysts in *Pkd-1*-targeted heterozygous mice supports the role of somatic mutations in cyst development, but the *Pkd2* mutant mouse, which also closely mimics the human disease, has provided confirmation that somatic loss of *Pkd2* expression is both necessary and sufficient for renal cyst formation [59, 60]. A mutant exon 1 was introduced in tandem with the wild-type exon 1, forming an unstable allele that was shown to undergo somatic inactivation by intragenic homologous recombination to produce a null allele. Mice heterozygous and homozygous for the mutation developed PKD. It should now be possible to determine other factors that influence disease expression, as these two mouse models mimic the human disease.

Tissue expression of polycystin-1

Since the cloning of the PKD1 gene in 1994 and the characterisation of the complete complementary DNA (cDNA) in 1995 there have been many reports examining the tissue expression of the PKD1 gene and the immunolocalisation of polycystin-1. Despite some discrepancies in the reported expression of this gene and its protein, a consensus is now beginning to emerge about its developmental and tissue-specific expression pattern. Analysis of RNA expression by Northern blotting and RNase protection assay has shown the PKD1 gene is widely expressed in various cell lines and fetal and adult tissues, with highest levels being found in brain and testes [31, 61].

Immunodetection of polycystin-1 has been reported by Western blotting, conventional and fluorescent microscopy, and electron microscopy, providing data on tissue distribution and subcellular localisation. Most antibodies have been raised against epitopes in the C-terminus of the protein, usually in the cytoplasmic tail. Using Western blot analysis, several antibodies recognise a distinct high molecular weight protein of the predicted size $\sim$460 kDa [62]. More typical is the detection of multiple molecular weight species that have been interpreted as proteolytic degradation products. The analysis and characterisation of full-length polycystin-1 is therefore likely to be a difficult technical challenge. Immunoprecipitation of intact polycystin from human tissues has not been described.

The tissue localisation of polycystin-1 has been explored in frozen and fixed fetal, adult and ADPKD tissues [61–68]. Several different antigen unmasking techniques have been reported to be necessary to achieve staining, but even allowing for these differences in analysis, polycystin-1 is widely expressed during fetal development and at consistently higher levels than in adult tissues.In fetal kidney polycystin is expressed at high levels in the ureteric bud and ureteric bud-derived structures such as collecting ducts. Staining has been reported to be weaker at the tips, where induction of mesenchymal-epithelial transform occurs in the metanephric mesenchyme [62, 65]. Uninduced mesenchyme is negative, with weak staining restricted, in most reports, to the comma and tubular portions of the S-shaped bodies, structures which differentiate into proximal and distal tubules. Occasional staining of the glomerular parietal epithelium is reported, but other structures such as glomeruli and the vasculature are negative. In later stages of gestation tubular staining appears to be most consistent in distal tubules and collecting ducts, although low levels have been seen in proximal tubules. These observations are consistent with the origin of cysts in ADPKD from all regions of the nephron.

Other fetal tissues that express polycystin-1 include liver and pancreas, cardiac muscle, skin, lung, bowel, gonads and adrenal cortex [63, 69, 70]. In the liver and pancreas staining is mainly confined to bile duct and pancreatic duct epithelial cells and their precursors.

In adult tissues staining is consistently seen at lower levels than in fetal tissues. In adult kidney polycystin-1 expression is seen most consistently in glomerular parietal epithelial cells and the distal part of the nephron, especially the collecting ducts (fig. 3). Glomeruli and vascular structures are usually reported as negative, but endothelial, podocyte and basement membrane staining have all been occasionally reported [61, 62, 64–66]. Increased polycystin expression has been reported in the renal proximal tubules in the regenerative recovery phase of ischaemic injury with a return to undetectable levels on recovery [64]. This suggests a role in epithelial cell proliferation and differentiation and should prompt a search for polycystin expression in other renal disorders.

In ADPKD tissues a consistent finding with all antibodies is the increased staining of cyst lining epithelial cells [61–68]. A small and variable subset of cysts do not stain, a phenomenon also seen in mouse ADPKD, providing further support for additional somatic null mutations being involved in cyst formation [59].

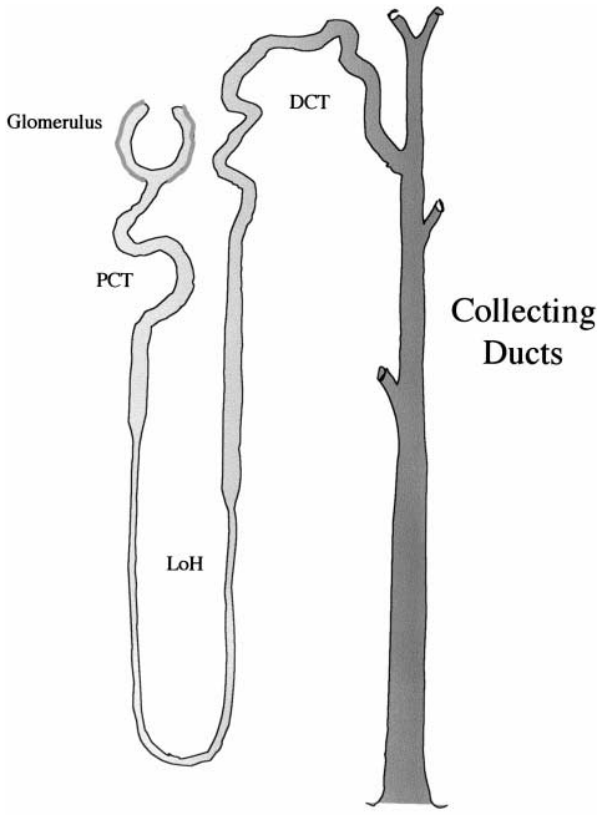

Figure 3. Polycystin-1 is predominantly expressed in the distal nephron. In this schematic representation of polycystin-1 expression in the adult nephron, maximal expression is seen in the distal convoluted tubule (DCT), collecting ducts and glomerular parietal epithelium adjacent to the proximal convoluted tubule (PCT). All regions of the nephron have been reported to express polycystin with lower levels in the PCT when seen.

Bile duct and pancreatic duct staining is also present in adult tissues, both sites of cyst formation in ADPKD [62, 64]. Epithelial cell staining has also been observed in breast, skin and cervix. Other sites of expression that have been reported include endothelial cells, cardiac myocytes and vascular smooth muscle cells [63, 64, 68, 71]. As cardiovascular manifestations are common in ADPKD, the lack of consistent staining in the vasculature has been surprising. However, Griffin et al. have described expression in vascular smooth muscle cells in adult elastic arteries using a technique of antigen retrieval combining microwaving with nonspecific protease or elastase digestion [71].

Whilst this description of polycystin expression represents a consensus from the many published reports, there is still a need for a wider description of polycystin expression in fetal and adult tissues to achieve a final consensus. Localisation in other species, especially mouse, and studies of polycystin-2 expression may also help to resolve some of the current discrepancies.

In studies of murine polycystin-1 expression two different antisera that recognise a high molecular weight protein have been used to stain fetal and adult tissues [72, 73]. In the study by Geng et al. expression was highest in fetal and early postnatal life, falling rapidly to low adult levels as determined by Northern and Western blot analysis. Staining of fetal kidneys revealed a restricted pattern of expression that was confined to the ureteric bud, collecting ducts and renal pelvis. No expression was detected in mesenchyme-derived structures at early (comma and S-shaped bodies) or late stages of tubulogenesis [73]. Immunostaining was identical from E18 kidneys until 1 week postnatal when levels of staining in the collecting ducts fell to low levels maintained during adulthood. In other tissues expression was confined to epithelial cells except in myocardium and was seen in bile ducts, pancreatic ducts, bronchioles, small intestine, and choiroid and ependyma of the brain.

Griffin et al. again reported expression in the ureteric bud but also in condensing mesenchymal cells. Epithelia in developing tubules also developed strong staining. In postnatal and adult, kidney staining was observed in proximal and distal tubules, collecting ducts and glomerular parietal epithelial cells. In cultured mouse metanephroi staining was present in the ureteric bud, comma and S-shaped bodies. In nonrenal tissues expression was seen in hepatocytes and biliary epithelium, brain, choroid plexus, cardiac myocytes, intestinal epithelial cells, bronchial epithelium and skeletal muscle [72].

Thus in murine tissues there is again some discordance of expression patterns between different antibodies as seen in human tissues. Some limited data on the expression of polycystin-2 is available from mouse kidney which demonstrates localisation to the medullary thick ascending limbs of Henle, distal convoluted tubule, and cortical and medullary collecting ducts with strongest staining in the medullary thick ascending limbs of Henle [60]. Weak staining was also seen in the proximal convoluted tubule (PCT). No glomerular, interstitial or vascular staining was noted. If one assumes a direct interaction between polycystin-1 and polycystin-2, these observations would seem to support polycystin-1 expression in all parts of the nephron, except the glomerulus. Different antibody affinities and antigen-unmasking techniques are therefore likely to explain the differing patterns of polycystin-1 expression seen between different reports. However, strongest expression of polycystin-1 is most often reported in collecting ducts. Further descriptions of polycystin-2 expression in humans and mouse are still awaited, but unpublished data from our own laboratory demonstrate strong basolateral expression of polycystin-2 in human and murine renal epithelial cells, mainly in the medullary thick ascending limbs of Henle and DCT, with weak staining in collecting ducts in a pattern identical to that reported by Wu et al. [60]. Although both proteins are therefore localised to the same regions of the nephron, the level of expression of polycystin-2 appears to contrast with that of polycystin-1. Indeed, staining of adjacent sections of renal cortex demonstrates colocalisation of polycystin-1 and polycsytin-2 only to a subset of tubules of DCT morphology (fig. 4). It is therefore possible that some functions of the polycystins in vivo are independent of their direct interaction at the cell surface.

Subcellular distribution

Many of the predicted structural domains of polycystin-1 are known to be extracellular [28, 34]. The presence of up to 11 transmembrane domains defines a large extracellular N-terminal region and a small cytoplasmic C-terminal tail. Localisation studies would therefore be expected to identify an integral membrane protein. Although immunohistochemistry lacks the resolution to accurately determine the precise subcellular localisation of proteins, several reports have described staining with membrane accentuation. Biochemical studies have also shown polycystin-1 to be localised in detergent-extractable membrane fractions [62]. More definitive localisation studies have used laser scanning confocal microscopy and immunoelectron microscopy [62, 64, 74]. Polycystin-1 has been localised to the lateral cell membranes in both cultured epithelial and endothelial cells and adult renal epithelial cells in vivo [64] (fig. 4). In epithelial cells of the ureteric bud and fetal kidney, expression is seen on all membrane surfaces, which may reflect differences in the state of cellular differentiation or different functions of polycystin in different cell types at different stages of development [75]. Expression has also been localised to the points of cell-cell contact in a human erythroleukaemia cell line, K562, and renal epithelial cell line, KJ29 [76]. Expression was inversely correlated with the state of differentiation.

Polycystin interactions

Although the severity of ADPKD may differ depending on whether mutations are in PKD1 or PKD2, the disease phenotype is indistinguishable [13, 77]. This has led to the suggestion that polycystin-1 and polycystin-2 may interact directly together, associate with the same protein(s) or be involved in different stages of the same cellular signalling pathway [27]. The structure of polycystin-1 predicts a protein with multiple extracellular domains and a short C-terminal cytoplasmic tail, whilst polycystin-2 is predicted to have N- and C-terminal cytoplasmic regions and homology to a voltage-activated Ca^{2+} channel [27, 28]. This suggests that extracellular ligands of polycystin-1 may lead to alterations in intracellular ion concentrations through direct interaction with polycystin-2. Two independent papers provide compelling evidence to support this hypothesis [78, 79]. Both groups observed the presence of potential coiled-coil domains in the C-termini of each protein. These α-helical domains are able to interact with other proteins containing coiled-coil domains and non-coiled-coil structures and were potential sites of polycystin-1 and polycystin-2 homotypic and heterotypic interactions. The predicted coiled-coil domain of polycystin-1 is conserved in the Fugu PKD1 gene lending support to its functional role [35].

Qian et al. used the yeast two-hybrid system to investigate possible polycystin interactions [78]. This demonstrated a direct interaction between the C-terminus of polycystin-1 and polycystin-2 and a homotypic interaction between the polycystin-2 C-terminus. The interactions were shown to be dependent on the presence of the coiled-coil domains using both naturally occurring and engineered mutations in critical structural residues. These results were confirmed using an in vitro binding assay that also demonstrated the preferential formation of polycystin-1 and polycystin-2 homotypic interactions. They therefore concluded that these proteins were able to undergo homo- and heterotypic interactions mediated by the predicted coiled-coil domains, so strengthening the case for the polycystins forming a membrane-associated protein complex.

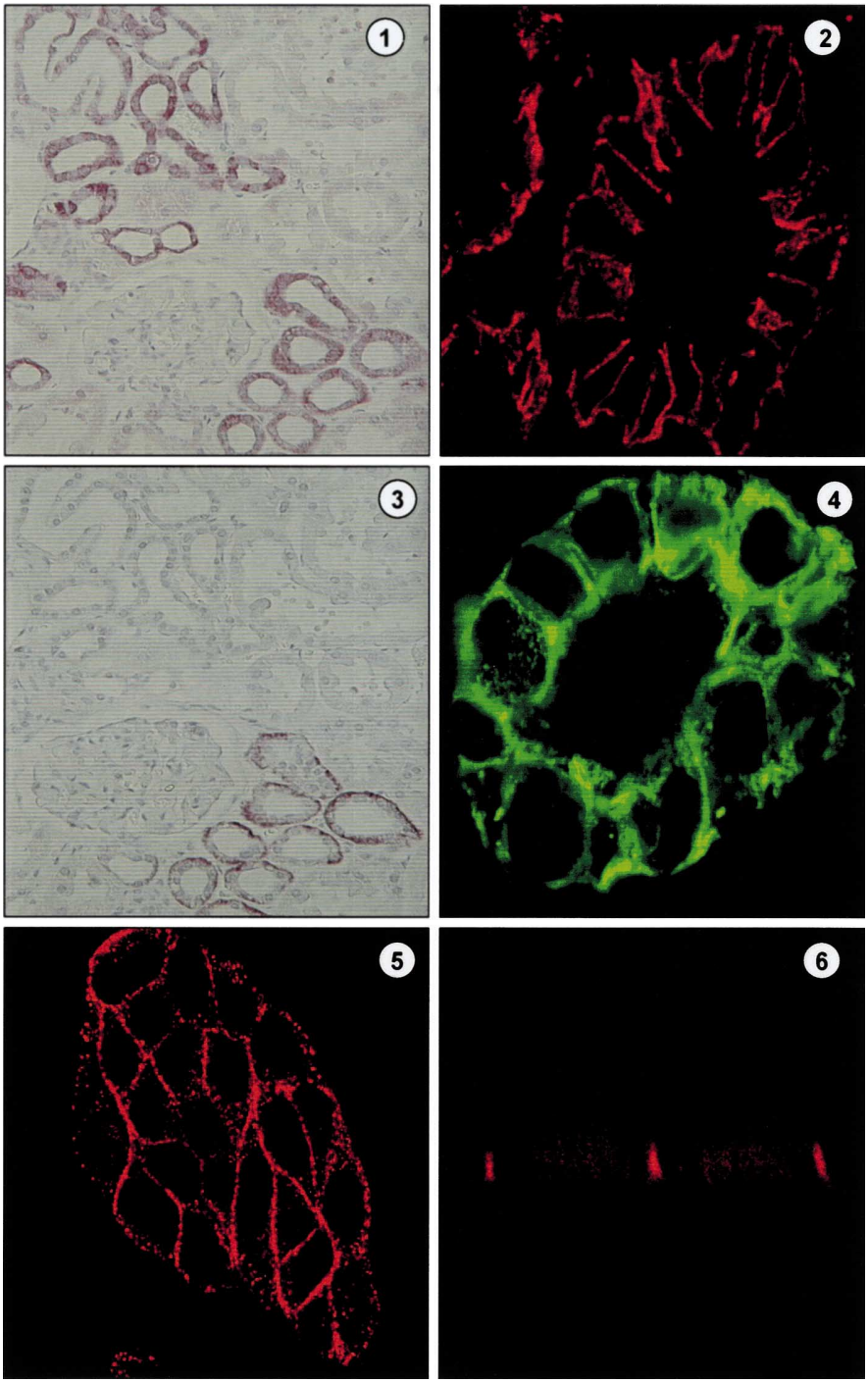

Figure 4. Expression of polycystin-1 and polycystin-2 in human kidney and MDCK cells. (*1*) Paraffin-fixed sections of adult human kidney cortex stained with anti-polycystin-1 antibody anti-FP-LRR [64] indicate that polycystin-1 is expressed predominantly in the distal tubules and collecting ducts (× 20, VIP, Vector Laboratories). (*2*) Immunofluorescence microscopy of individual tubules stained with the same antibody reveals the cell membrane localisation of polycystin-1 with accentuation at the lateral cell junction. (*3*) Paraffin-fixed sections of adult human kidney cortex adjacent to (*1*) stained with anti-polycystin-2 antibodies [unpublished observations] reveals a basolateral distribution in distal tubules (× 20, VIP, Vector Laboratories). All tubules that stain for polycstin-2 also stain for polycystin-1, whilst some polycystin-1-positive tubules are negative for polycystin-2 at this level of detection. (*4*) Immunofluorescence microscopy of individual tubules stained with anti-polycystin-2 shows this protein also to be distributed to the cell membrane. Cultured MDCK cells stained with anti-FP-LRR antibody and other anti-polycystin-1 antisera [64] (data not shown) and examined by confocal immunofluorescence microscopy in the horizontal plane (*5*) and the vertical plane (*6*) further demonstrate the junctional localisation of polycystin-1.

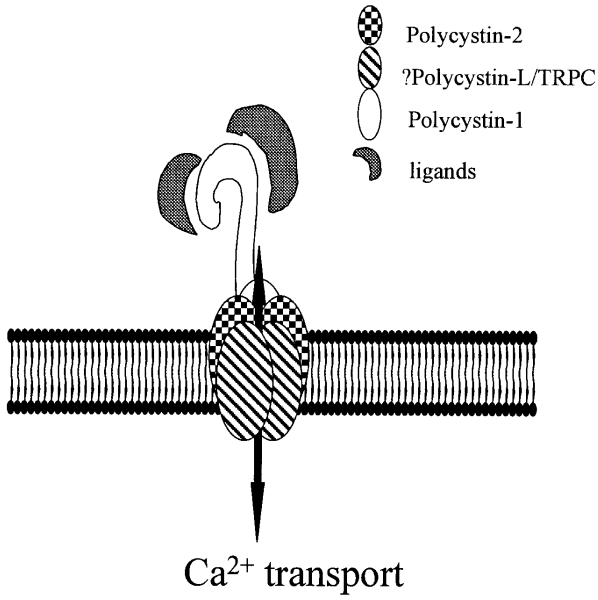

Figure 5. A schematic representation of the possible interactions between polycystin-1 and polycystin-2 at the plasma membrane of renal tubule epithelial cells. Polycystin-1 and polycystin-2 may interact together or with additional protein subunits, such as PKDL or members of the TRPC family, to form a large membrane Ca^{2+} ion channel regulated by extracellular polycystin-1/ligand interactions.

Tsiokas et al. also used the yeast two-hybrid system to explore polycystin interactions [79]. Their data also identified polycystin-1 and polycystin-2 homo- and heterotypic interactions. Potential coiled-coil structures were shown to mediate polycystin-1-polycystin-2 interactions and polycystin-2 homodimerisation. However, the site of interaction of polycystin-2 with polycystin-1 is located outside of the coiled-coil domain. Therefore, polycystin-2 interacts with polycystin-1 through a domain that is separate from its homodimerisation domain. These data were also confirmed in in vitro and in vivo immunoprecipitation assays. However, no evidence for polycystin-1 homodimerisation was found. Strikingly, polycystin-1 expression was observed to be increased in the presence of polycystin-2, suggesting that polycystin-1 stability is dependent on the presence of polycystin-2 either to stabilise membrane complexes or prevent degradation.

These studies, together with preliminary coimmunolocalisation data that demonstrate expression in the same part of the renal tubule, collectively suggest that polycystin-1-polycystin-2 heterodimers and polycystin-2 homodimers may form part of a multimeric protein complex involved in functions that control epithelial cell differentiation (figs 4 and 5). Mutations in either PKD gene that interfere with these interactions by reducing the amount of functional protein or abolishing the interaction domains will therefore destabilise the protein complex, leading to altered epithelial cell function and cyst formation.

The C-terminal region of polycystin-1 has also been shown to activate the transcription factor, AP-1, and c-Jun N-terminal kinase (JNK) [80]. This effect is dependent on its membrane localisation and is mediated by protein kinase C α (PKCα) and Rac-1 and Cdc42, two members of the small guanine triphosphate (GTP)-binding protein family, Rho. The other components of this signalling cascade remain to be identified. AP1 (activating protein-1) is a term that describes dimeric transcription factors composed of Jun, Fos or ATF (activating transcription factor). They bind to a common AP1 DNA binding site and regulate gene transcription, and thus many diverse biological functions including cell proliferation, differentiation and apoptosis. Abnormalities in all these processes have been reported in ADPKD, so that contributions from altered gene transcription and ion channel function mediated by abnormal polycystin protein complexes may have a central role in cystogenesis. Arnould et al. also postulated that abnormal Rho activation mediated by polycystin-1 in ADPKD could explain some of the abnormalities of cell polarity and protein sorting that have been previously reported, as Rho has been shown to regulate the actin cytoskeleton and cell shape and adhesion. The definition of some of the intracellular binding partners of the polycystins now points to the need to identify extracellular ligands. Both polycystin-1 and polycystin-2 may participate in extracellular binding, but the domain structure of polycystin-1 favours its role in mediating these interactions. So far no extracellular ligands have been identified.

The impression is therefore that the polycystins may form part of a large multimeric membrane protein complex that regulates, in response to a wide range of extracellular signals, gene transcription and transmembrane ion flux. A single function for these proteins looks extremely unlikely.

New insights in to polycystin function

During the 1998 American Society of Nephrology meeting in Philadelphia, many exciting new observations were reported about the biology of the polycystins, some of which have now been published in peer-reviewed journals. Many of these added support to the hypothesis that the polycystins form large membrane complexes involved in adhesion-mediated signal transduction. Potential interactions with focal adhesion complex proteins vinculin and paxillin and the actin-binding

proteins actinin and talin support a role in communication between the extracellular compartment and the actin cytoskeleton [81]. Other parts of this signalling cascade demonstrated experimentally include activation of Wnt signalling and interactions with heterotrimeric G proteins [82, 83]. Further evidence for a role in Ca^{2+} flux was demonstrated by the in vitro interaction of the C-terminal regions of polycystin-1 and polycystin-2 with members of the TRPC (transient receptor potential channel) family [84]. Their role in maintaining intracellular Ca^{2+} stores suggests that one mechanism underlying cyst formation is mutations in PKD1 and PKD2 causing cellular Ca^{2+} depletion. The development of mouse models of ADPKD will greatly enhance the ability to examine these potential interactions in vivo. Both *Pkd1* and *Pkd2* mutations recapitulate the disease in mouse, and a cystic phenotype has also been described in a mouse PKD1 transgenic line [59, 60, 85, 86]. Whilst most of the novel data presented concerned the role of the intracellular portions of the polycystins, the extracellular ligands of polycystin-1 remain unidentified.

Future research will concentrate on the development of further in vivo and in vitro models to examine polycystin function and define further intracellular and extracellular ligands. Technical difficulties in the expression and study of such a large protein as polycystin-1 also present a major challenge. Once all of the components of the polycystin pathway have been identified, its role in a diverse range of cellular processes and the pathogenesis of ADPKD should become much clearer. Scientists, clinicians and patients alike keenly wait whether this will lead to the development of any therapeutic strategies for ADPKD.

Conclusions

The polycystins are a novel protein family whose precise function remains to be determined. Evidence is beginning to accumulate to suggest that they may associate together as a multifunctional membrane-associated complex in an adhesion-mediated signal transduction pathway, although they may be able to function independently of each other. Direct effects on intracellular Ca^{2+} levels, AP-1 activation, G protein-coupled signalling and the actin cytoskeleton may all contribute to the altered cellular differentiation, abnormal proliferation and apoptosis seen in ADPKD. Other components of this complex or pathway may therefore be involved in the pathogenesis of other renal cystic and developmental abnormalities. Identification of these extracellular and intracellular interacting molecules will define this signalling pathway and identify one of the key pathways involved in renal epithelial cell development. The tantalising speculation that ADPKD results from abnormalities in an ion channel suggests that pharmacological manipulation may provide a valuable way of modifying the clinical progression of this common disease.

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