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Autosomal dominant polycystic kidney disease: clues to pathogenesis

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Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutation of one of two genes: *PKD1* (16p13.3) or *PKD2* (4q13–23). *PKD1* accounts for ~85% of pedigrees and is associated with significantly more severe cystic disease. The ADPKD genes encode proteins, polycystin-1 and polycystin-2, which are very different in size and structure, but which have a region of homology and may interact as part of the same complex. Polycystin-1 is a large, integral membrane protein (~460 kDa) predicted to be involved in cell–cell and/or cell–matrix interactions. Polycystin-2 (~110 kDa) is related to polycystin-1 and voltage-activated and transient receptor potential channel subunits, suggesting that the polycystins may also be associated with ion transport. A polycystin complex could regulate cellular events (that are abnormal in ADPKD) in response to specific extracellular cues, mediated by controlling cellular Ca^{2+} levels and/or other signalling pathways. Recently, two further polycystin-like molecules have been identified, indicating roles for this novel protein family beyond the kidney. A wide range of different mutations to the *PKD1* or *PKD2* gene have been detected, most predicted to truncate and inactivate the proteins. A somatic second hit may be required for focal cyst development, although there is widespread immunohistochemical evidence of polycystin expression in cystic epithelia. Disruption of the mouse *Pkd1* gene leads to death in the perinatal period with massive cystic expansion in homozygotes and age-related cyst development in heterozygotes. Normal renal development in $Pkd1^{del34/del34}$ mice up to embryonic day ~15.5 suggests a role for polycystin-1 in developing and maintaining the tubular architecture, consistent with the localization of the protein, rather than nephron induction. Renal cystic disease in homo- and heterozygotes of a Pkd2 mouse model with a disrupted exon 1 inserted in tandem with the normal exon (and prone to somatic recombination, which inactivates the gene) supports a role for somatic events in cystogenesis.

INTRODUCTION

The autosomal dominant form of polycystic kidney disease (ADPKD) is one of the most common monogenetic disorders (frequency ~0.1%). The disease is characterized by the progressive development and expansion of renal cysts, which ultimately completely replace the precise architecture of the kidney and result in end stage renal disease (ESRD). Analysis of the early stages of cyst development shows that they develop as dilations from any part of the nephron. The dilations are associated with a thickened and disordered basement membrane and lined with a single layer of epithelial cells which show increased proliferation, protein sorting defects and polarity changes (1,2). ADPKD is not just a disease of the kidney; cysts are also seen in many other ductal structures, including the liver and pancreas. A number of other abnormalities are also associated with ADPKD, including an increased frequency of intracranial aneurysms, which can result in subarachnoid haemorrhage, and heart valve defects (3). These changes indicate that the basic defect in this disorder is not limited to the kidney, but affects many epithelial and non-epithelial structures. In recent years progress towards understanding this complex disorder has been made using the genetic method of positional cloning to identify the primary defect(s).

ADPKD IS GENETICALLY HETEROGENEOUS

An early finding from genetic linkage analysis was that more than one gene causes ADPKD. The majority of pedigrees [~85% in European populations (4,5)] are linked to a locus in chromosome region 16p13.3, now designated *PKD1*, while most of the remainder map to 4q13–23, *PKD2*. A small number of unlinked families have been described, suggesting the presence of at least one further locus (6,7). Phenotypically, PKD1 and PKD2 appear very similar; however, there is an important difference in disease severity, with ESRD occurring on average ~16 years earlier in PKD1 [PKD1, 53 years; PKD2, 69.1 years (8)].

THE ADPKD GENES

The *PKD1* gene was identified due to a chromosomal translocation that disrupted the gene in one family (9). The transcript is large (14 136 bp) and encoded by 46 exons in a genomic region of ~50 kb (10,11). The *PKD1* genomic region is complex, with the area encoding the 5′ region of the gene to exon

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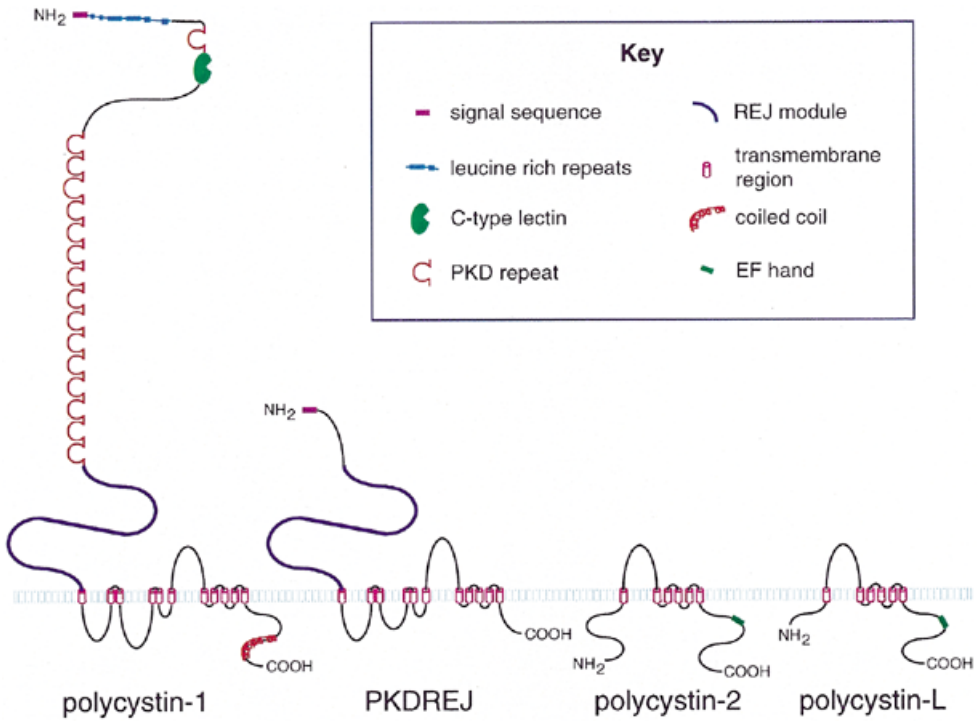

Figure 1. Diagram showing the proposed structures of various members of the polycystin protein family. See key for details of structural motifs. Only polycystin-1 and -2 are associated with ADPKD. All of the proteins show sequence similarity that covers the TM regions of polycystin-2 and -L (the product of *PKDL*) and the last six TM domains of polycystin-1 and PKDREJ.

33 reiterated several times elsewhere at a site more proximal on chromosome 16 (9). This reiterated region encodes a number of *PKD1*-like, homologous genes (*HG*) that have significantly complicated mutation analysis. The second ADPKD gene, *PKD2*, was identified from candidates in the linkage defined interval by sequence similarity with PKD1 (12). The *PKD2* transcript is ~5.4 kb and encoded by 15 exons in a genomic area of ~68 kb (12,13).

THE ADPKD PROTEINS

The protein products of the *PKD1* and *PKD2* genes, polycystin-1 and -2, respectively, have been predicted from cDNA sequence. Polycystin-1 is a large protein (4302 amino acids, 460 kDa) associated with the cell membrane, with 11 transmembrane (TM) passes predicted (Fig. 1) (14). The likely structure of the protein has been refined by comparative analysis with orthologues, especially of the puffer fish, *Fugu* (14). The protein has a large extracellular portion containing a number of recognized motifs including: two leucine-rich repeats; a C-type lectin; 16 copies of a unique PKD domain; and a region of homology with a sea urchin protein, the receptor for egg jelly (suREJ). Recently, the structure of the PKD domain was resolved by NMR analysis and this showed a β-sandwich, similar to an Ig-like fold, but thought to have a distinct origin (15). The area of homology with the suREJ protein extends over ~1000 amino acids from the PKD domains to the first TM domain (16). The suREJ protein is located on the sperm head and involved in triggering the acrosome reaction, an exocytic remodelling of the acrosomal membrane, on contact with the glycoprotein coat of the egg (17). The acrosome reaction is an essential step in the fertilization process and mediated by an influx of Ca^{2+} ions. The sequence similarity to the REJ module in polycystin-1 suggests that this region of the protein may also play a role in regulating ion transport.

Analysis of polycystin-2 also suggested a role in ion transport. The 968 amino acid (~110 kDa) polycystin-2 is predicted to contain six TM domains, with cytoplasmic N- and C-termini (Fig. 1). It has significant sequence homology with Ca^{2+} and Na^{+} voltage-activated (VAC) (12) and transient receptor potential (TRP) channel subunits (18), as well as with polycystin-1. The corresponding homologous area of polycystin-1 comprises the last six TM regions (Fig. 1) (14). TRP channel subunits consist of six TM domains (19) and a variety of different homo- and heteromultimers of TRP proteins form channels similar in structure to VACs (20), which consist of four subunits each containing six TM domains (21). The structure of the polycystin proteins suggests that they may also form a functional channel as homo- and/or heteromultimers. *In vitro* evidence of an interaction between a coiled coil domain in the cytoplasmic tail of polycystin-1 and part of the C-terminal tail of polycystin-2 has been described (22,23). Recently, co-immunoprecipitation of polycystin-2 and the TRP protein TRPC1 has been shown, after transient co-transfection of HEK293T cells with full-length, tagged constructs, suggesting the formation of a heterogeneous channel consisting of TRP and polycystin subunits (18). As well as interacting with polycystin-2, the cytoplasmic tail of polycystin-1 has also been shown to bind heterotrimeric G proteins *in vitro*, suggesting a role in signal transduction (24). Further support for a role in signalling comes from evidence that the C-terminal region of polycystin-1 may induce AP-1 transcription through activation of protein kinase C and c-Jun terminal kinase and has also been implicated in

modulating Wnt signalling (25,26). The C-terminal tail of polycystin-2 contains a Ca^{2+}-binding EF hand (12).

In summary, the proposed extracellular part of polycystin-1 contains a number of motifs usually involved in protein–protein or protein–carbohydrate contacts, suggesting a role in cell–cell or cell–matrix interactions. Extracellular cues may be transmitted through one or more signalling pathway, regulating the multiple cellular processes that are defective in ADPKD. The signalling could involve Ca^{2+} transport, polycystin-1 and -2 may be components of a ligand-gated ion channel. Ca^{2+} is a ubiquitous second messenger and abnormal Ca^{2+} regulation may trigger the cellular changes associated with cystogenesis.

EXPRESSION OF THE POLYCYSTIN PROTEINS

Analysis of *PKD1* and *PKD2* mRNA showed expression in practically every tissue tested, showing that both genes are widely expressed (12,27). Many different polycystin-1 antibodies have now been described and, although there is some variation, a consensus for the expression pattern is emerging. Expression generally appears higher during development, with more localized expression in the adult. In the fetal kidney expression is seen in tubular epithelia of maturing tubules, with only weak expression in the nephrogenic zone of the developing kidney (27–31). In the adult, expression appears more localized to collecting ducts and distal tubules (32–35). Outside the kidney, polycystin-1 is expressed in fetal ductal epithelia in the liver, pancreas, lung, breast and reproductive organs (28,32,34–36). In addition, polycystin-1 expression has been noted in fetal endothelia and smooth muscle of blood vessels (28,34,35), cardiac and skeletal muscle (29,35–37) and in the brain (31,34–37). The pattern of expression outside the kidney reflects the systematic nature of the disease and suggests a direct role in the associated abnormalities. Descriptions of polycystin-2 antibodies are limited so far, but Ong *et al*. (35) have demonstrated a very similar expression pattern to polycystin-1, both within and beyond the kidney, consistent with the notion that they may form part of the same complex.

In contrast to the cellular localization, there is little consensus on the subcellular localization of polycystin-1. Although several western blot studies have shown localization to membrane-enriched fractions (29,32,33,38), immunolocalization has been inconsistent. Examples of plasma membrane localization have been described as: mainly apical (32); mainly basal (31); all cell membranes in the nephrogenic zone but cytoplasmic later (33); and membrane staining of collecting ducts and ductal tissue, with lateral staining of cultured endothelial cells (34). Other studies have shown mainly cytoplasmic staining (27,28,30), with only occasional evidence of clear membrane association (35). The present uncertainty may reflect genuine differences in localization during development and in different tissues, with the possibility that the protein is continuously recycled with appropriate membrane targeting dependent on interaction with other members of a polycystin complex and/or on specific extracellular interactions.

THE POLYCYSTIN FAMILY OF PROTEINS

Recently, the description of two genes encoding polycystin-like proteins has revealed the existence of a polycystin protein family. The first to be described, *PKDL*, encodes a polycystin-2-like molecule with a similar predicted topology and TM region (Fig. 1) (39,40). The expression pattern of this gene is not yet clearly resolved, although it does not appear to be widely expressed like the ADPKD genes; the isolation of ESTs from retina may be significant. Analysis of unlinked ADPKD families has shown no evidence that *PKDL* is associated with this disorder (39,40). The second gene to be described, *PKDREJ*, also does not appear to be ADPKD related, as it is expressed exclusively in mature testis (41). The predicted PKDREJ protein is similar to polycystin-1 over ~2000 amino acids, including the REJ module and the entire TM region (Fig. 1). The expression pattern and structure of this protein suggests that it may be functionally related to the suREJ protein and hence play a role in human fertilization. The acrosome reaction is triggered by an influx of Ca^{2+} (42) and it is possible that, akin to polycystin-1 and -2, the TM section of PKDREJ may form part of a Ca^{2+} channel, as a homo- or heteromultimer with as yet undiscovered subunits. The polycystin protein family appears to have a range of functions in diverse tissue types. A central finding, however, is the possible association with Ca^{2+} transport.

MUTATIONS OF THE ADPKD GENES

Screening for mutations of the *PKD1* gene has been complicated by the genomic structure of the region encoding the 5′ 80% of the gene. Consequently, the number of reported mutations is limited, with many in the 3′ single-copy region (43–52). Nevertheless, parts of the duplicated region have been screened for mutations with a variety of different strategies including: a primer anchored in the single copy region to provide *PKD1* specificity (53–55); using primers that match the rare differences between the *PKD1* and *HG* sequences (56,57); and without specifically using the protein truncation test (58). No full screen of the gene has yet been described, but some conclusions can be drawn from the ~50 mutations now described. Most mutations are unique to a single family and, even on the rare occasion that the same mutation has been found more than once, there is evidence that the change is recurrent (50). This indicates that a significant rate of new mutation is occurring at *PKD1*. It has been suggested that the level of mutation at *PKD1* is enhanced by instability caused by a large polypyrimidine tract in IVS 21 (54) or by gene conversion events involving the *HG* sequence (57). However, although these may be significant factors, they do not appear to account for the majority of *PKD1* mutations (53,56).

Analysis of the 15 exons of the *PKD2* gene has proved more straightforward, with ~30 mutations now described (12,59–63). The mutations are spread throughout the gene and, apart from one missense change, are nonsense, frameshifting insertions or deletions or splicing changes which are predicted to inactivate the gene. Analysis of *PKD1* shows a similar picture, with most mutations predicted to truncate the product due to nonsense or frameshifting changes, although a number of in-frame and possible missense mutations have been described (46,51–53,55).

PKD1/*TSC2* DELETIONS

The suggestion that ADPKD mutations are inactivating is supported by a rare group of patients that have early onset polycystic kidney disease and a second genetic disease, tuberous sclerosis (TSC). A major TSC gene, *TSC2*, lies immediately adjacent to *PKD1* on chromosome 16 (64). Analysis of TSC patients with severe PKD has shown that many have deletions that disrupt *PKD1* and *TSC2*, often completely deleting *PKD1* (65–68), indicating that a null *PKD1* allele is associated with cystogenesis.

The PKD in these cases is usually much more severe than in typical PKD1 patients, suggesting a synergistic role for polycystin-1 and the TSC2 protein, tuberin, in cyst development.

THE MUTATIONAL MECHANISM IN ADPKD

A major point of discussion has been why cysts only develop in a proportion of nephrons when all cells have the same germline mutation. It has been suggested that cyst development is a two-hit process, like inherited cancers, with a somatic second mutation to the ADPKD gene required before a cyst can develop (69). Support for this theory has come from DNA analysis of cystic epithelia isolated from single cysts, with clonality of individual cysts and loss of heterozygosity (LOH) of microsatellites from within or close to the *PKD1* gene demonstrated in ~15% of cysts (70,71). A further intragenic somatic mutation was found in one cyst and more recently LOH has been described in ~18% of cysts from a single PKD1 kidney (55), with LOH or intragenic mutations in 38% of liver cysts from two patients (57). Analysis of PKD2 cystic tissue from one patient showed that 43% of cysts had intragenic mutations, seven of nine of which had the same insertion (72). As predicted by the two-hit theory, in all informative cases, it is the wild-type allele that is somatically mutated.

Immunohistochemical analysis of ADPKD cystic epithelia has, however, generally shown strong staining with polycystin-1 antibodies (27,28,30,31,33), although in some cases variability and a proportion of negative cysts have been detected (32,34). Detailed analysis of cystic epithelia in PKD1 patients with defined truncating mutations, where only protein encoded by the normal allele should be detected, showed that the majority of cysts stained (27,38). Similar studies of PKD2 cystic epithelia with a polycystin-2 antibody have also shown staining of the majority of cysts (35). It is not yet clear how the two-hit hypothesis and polycystin staining of cystic epithelia can be reconciled. One possibility is that most somatic events are missense or in-frame, producing an inactivated protein that is detected with polycystin antibodies. The other possibility is that somatic mutations occur in the increasing cell number of the expanding cyst and are not required for cyst initiation. In this case a dosage reduction of the ADPKD protein, which, because it is required in strict molar ratios with other proteins, leads to the formation of functionless or dysregulated polycystin complexes.

TARGETED DISRUPTION OF *Pkd1* AND *Pkd2*

Further evidence that polycystin loss is associated with cyst development comes from mice with targeted disruption of the ADPKD orthologues. The *Pkd1* gene has been disrupted by deletion of exon 34, predicted to result in a frameshifting change (73). The homozygous animals die in the perinatal period with massively enlarged cystic kidneys and pancreas and pulmonary hypoplasia. Renal development appears normal up to embryonic day ~15.5, when cysts start to develop, suggesting, along with the polycystin-1 expression data, that the role of polycystin-1 is in establishing and maintaining the tubular architecture, rather than nephron induction. Renal cysts were not found in heterozygous animals aged <7 months (73), but have been detected in older animals (74). Interestingly, no liver cysts were found in the homozygotes but were found in the older heterozygotes.

Targeted disruption of *Pkd2* resulted in the production of an animal with a null allele (targeted in exon 1) and a second with a tandem insertion of the targeted exon (WS25), which is unstable, often recombining somatically to form a null allele (75). The null homozygotes die at embryonic days 13.5–15.5 and heterozygotes have a small number of cysts. *Pkd2*^{WS25} homo- and heterozygotes are viable but develop a renal cystic phenotype, apparently due to somatic recombination giving rise to null alleles. Analysis of cystic tissue by immunohistochemistry with a polycystin-2 antibody showed that the cystic tissue was negative, suggesting that complete loss of the protein is required for cyst development (75). This model further supports the role of somatic events in cyst development.

CONCLUSIONS

Inactivating mutations of either the *PKD1* or *PKD2* gene can lead to cyst development. The two polycystin proteins may form part of the same complex which is involved in detecting extracellular cues and transmitting these to the cellular environment, via a Ca^{2+} influx and/or other signalling pathway. Disruption of the polycystin complex in the disease state leads to dysregulation of the wide range of cellular processes that are abnormal in ADPKD.

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