the rate of renal progression in heavily proteinuric patients is extremely tight blood pressure control, as demonstrated by the modified diet in renal disease study[20].

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The polycystic kidney disease 1 (PKD-1) gene: an important clue in the study of renal cyst formation

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Solitary cysts are commonly found in normal kidneys, especially with increasing age. The hereditary renal cystic diseases, of which autosomal dominant polycystic kidney disease (ADPKD) is the most common (incidence 1 in 1,000 people), are however characterised by multiple cysts which are bilateral, often arise *in utero*, and lead to the progressive destruction of normal kidney tissue and gradual loss of renal function[1].

A major breakthrough in the study of this group of diseases came in 1985 when polycystic kidney disease 1 (PKD-1), the major gene responsible in almost 90% of patients with ADPKD, was linked to the short arm of chromosome 16 (16p)[2]. This was followed quickly by the recognition that at least 10% of ADPKD patients were not linked to chromosome 16, leading to the definition of further loci, PKD-2 (chromosome 4) and later PKD-3 (so far unlinked). Although the approximate position of PKD-1 was defined in 1985, the genomic arrangement at this locus was so complex that its precise location was not identified until 1994, by a combination of painstaking hard work and a decisive stroke of good fortune[3]. Subsequently, the PKD-1 gene and the protein it encodes, polycystin, have been the subject of intense investigation. During this period, PKD-2 has also been cloned and fully sequenced[4].

After pointing out the problems that continue to face researchers in this field, this article reviews the present state of knowledge of PKD-1 by addressing three specific questions:

1. What is known about the function of polycystin?
2. Can the marked phenotypic variability seen in individuals with PKD-1 be explained by differences in the position and type of mutations affecting the PKD-1 gene?
3. What is the molecular basis of cyst formation in PKD-1?

Problems in studying the PKD-1 gene

The PKD-1 gene has presented unique difficulties to researchers seeking to characterise it fully and study its function. At least two-thirds of PKD-1 lies in a genomic area duplicated more proximally on chromosome 16. This duplicate area encodes three genes with substantial (>97%) homology to PKD-1[3], illustrated in Figure 1 using the technique of fluorescent *in situ* hybridisation (FISH). These homologous genes all generate mRNA transcripts, but it is not known if these transcripts are translated into functional proteins. PKD-1 is a large gene, covering 52 kb of genomic DNA. It consists of 46 exons and encodes an mRNA transcript of 14 kb. Polycystin is thus predicted to have 4,302 amino acids with a calculated molecular weight of 460 kDa[5].

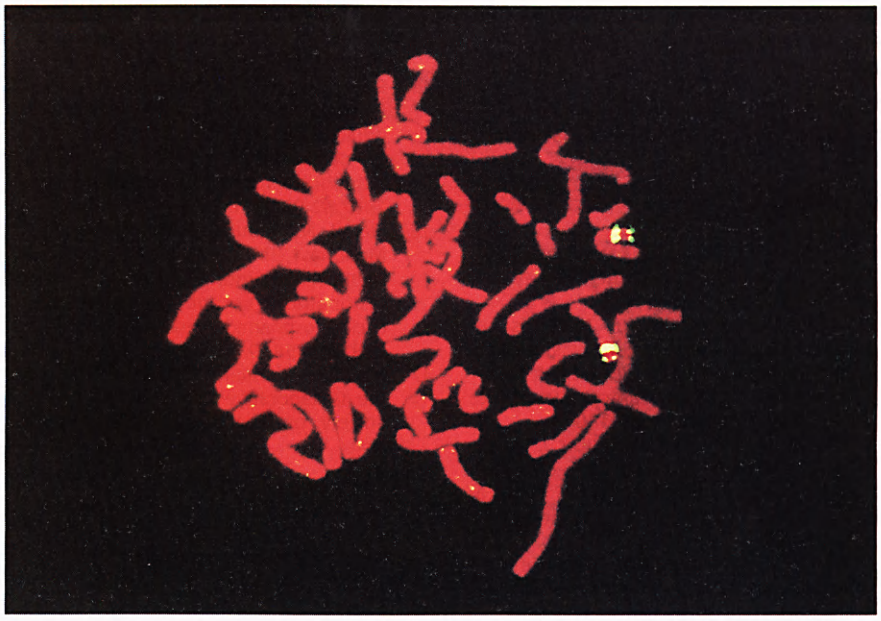

Figure 1. Fluorescent *in situ* hybridisation (FISH) of a biotinylated-cosmid from the duplicated area (CW10II) to a normal male chromosome metaphase spread. Duplication of this locus is illustrated by two sites of hybridisation on the short arm of chromosome 16 (16p). Note that the signal from the proximal site (duplicated area) is stronger than that from the distal (single-copy area). *Reproduced, with permission, from Ref 3.*

What does polycystin do?

The availability of the full gene sequence of PKD-1[5] has made possible a detailed comparison with other genes of known function. Figure 2 shows the most widely accepted model for polycystin. There is agreement that it is a membrane glycoprotein with multiple transmembrane domains. Two-thirds of the protein appears to be extracellular, leaving only a short intracellular tail. Polycystin is a novel protein unrelated to other known protein families. Nevertheless, it has a clear N-terminal signal sequence and several domains such as two leucine-rich repeats, a C-type lectin, and 16 immunoglobulin-like domains, extracellular motifs identified in other proteins involved in protein-protein interactions[5]. From these findings, polycystin would be expected to mediate cell-cell or cell-matrix interactions, either through homophilic (ie with itself) or heterophilic (ie with other, as yet unidentified, molecules) interactions.

More recently, part of the sequence of a 210 kDa protein found in sea urchin sperm, the REJ (receptor for egg jelly) protein, was shown to share extensive sequence homology with an extracellular portion of PKD-1[6]. Given that the REJ protein is thought to regulate ion channels, and also that the PKD-2 protein shares extensive homology with a family of voltage-activated calcium/sodium channels[4], it is possible that polycystin may also possess ion channel or ion channel regulating activity.

Recent studies have demonstrated that the PKD-1 gene is expressed in a wide range of adult human tissues, a finding consistent with the fact that ADPKD is a systemic renal disease[7]. Surprisingly, PKD-1 gene expression appeared to be highest in brain, and was detected only at moderate levels in the kidney. This study clearly demonstrates the need to delineate precisely the different cell types expressing polycystin in each tissue.

Immunohistochemical studies in the kidney using monoclonal antibodies raised to the unique intracellular tail of polycystin showed that renal epithelial cells were the major cell type expressing polycystin throughout the mature nephron[7]. Glomerular immunoreactivity was confined to the parietal epithelial cells of Bowman's capsule, close to the origins of the proximal tubule. In the developing human kidney, expression of polycystin was not observed in undifferentiated renal mesenchyme, but became evident in the earliest epithelial precursors (S-shaped and comma-shaped bodies) and in the distal branches of the ureteric bud, becoming more pronounced as these matured into tubular structures[7]. This relatively late expression pattern is more consistent with a role for polycystin in epithelial organisation (tubular formation) and/or the maintenance of epithelial differentiation, rather than in epithelial formation (ie induction).

A more intriguing finding was increased PKD-1 gene expression, as well as intense staining of the cystic epithelium for PKD-1 by immunohistochemistry, in adult PKD-1 kidneys[7]. This increase could reflect a reversion of cystic epithelial cells to a less differentiated ('fetal') phenotype. Alternatively, if the disease protein

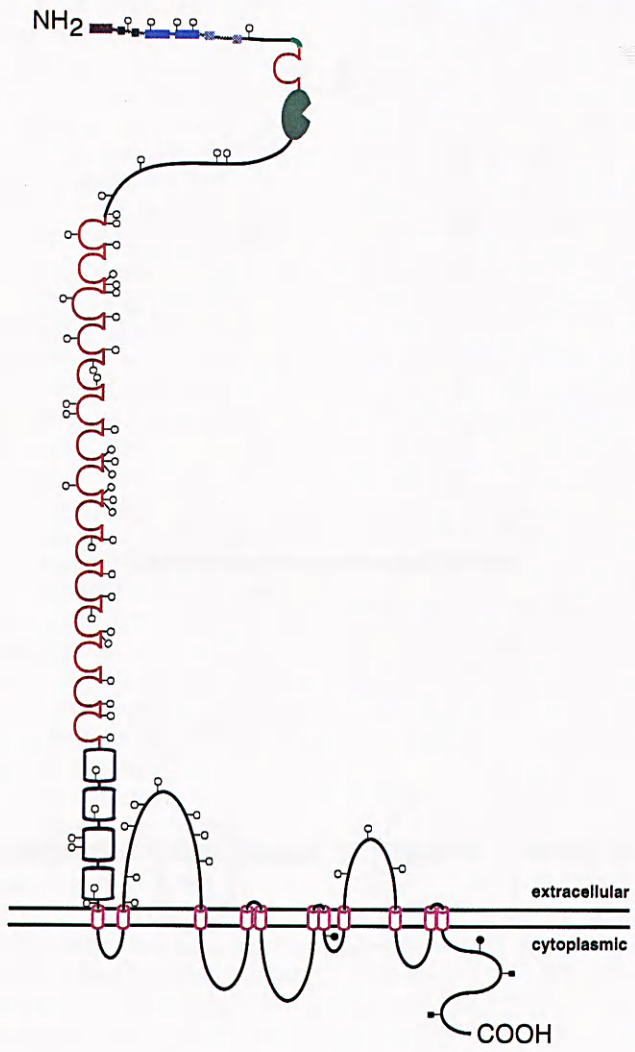

Figure 2. A proposed model for the PKD-1 protein, polycystin (see key for details). *Reproduced, with permission, from Ref 5.*

induces a functional cellular deficiency of polycystin, for example by binding to and inactivating the normal protein (see later), both genes may be upregulated if there is a negative feedback loop[7].

Genotype-phenotype correlations

The marked phenotypic heterogeneity in PKD-1 poses the important question whether any phenotype corresponds to any particular position or type of mutation. Such correlations would be of prognostic importance, but would also yield clues about the biological importance of different domains of the protein. Conversely, lack of any such correlation would point to the importance of other factors (genetic and/or environmental) in modifying the expression of disease.

Figure 3 illustrates some of the first mutations described within the unduplicated region of PKD-1[8]. So far, 25 mutations have been found in over half the coding exons (exon 22–46) of PKD-1 including, more recently, 11 mutations in the duplicated region (exon 22–32) of this gene[9]. The majority of these changes appear to be inactivating (ie they should lead to a non-functional disease protein); large deletions involving PKD-1 alone are however rare (but see later). Several missense changes have been found, but it has not been possible to obtain independent confirmation of their significance using a biological assay. With a few exceptions, each mutation appears to be unique for each family. Moreover, even where the same mutation has been found in more than one family, it seems to have arisen independently. Both these observations suggest a high rate of new mutation for PKD-1 in the population. To date,

no clear genotype-phenotype correlation has emerged for either the severity of renal disease or the incidence of extrarenal manifestations.

These studies have also confirmed previous observations of a high degree of intrafamilial variability in terms of the severity of renal disease. An extreme and interesting example of this is the phenomenon of *genetic anticipation*, in which a mildly affected parent gives rise to a severely affected child. At the genetic level, this has been associated with *dynamic mutations* which, in some diseases, are characterised by expansion of a trinucleotide repeat, either during or after meiosis. For PKD-1, however, the mutation appears to be stable, as seen in one family with early onset disease, and it seems highly likely that other modifying factors affect the expression of disease in each affected individual[10].

The mutational mechanism of cyst formation

PKD-1 is a dominantly inherited disease so, in theory, there should always be one normal PKD-1 allele present even in cystic cells. So how does the disease arise? This question is not academic, but has clear implications for devising appropriate treatment strategies. Any of three possible mechanisms could operate[11]:

- A *gain of function*. The disease protein could be toxic, induce a deleterious function, or interfere with the function of the normal protein, for example either by disrupting the interaction between two normal proteins or by competing for binding of a vital substrate.
- A *haplo-insufficiency*. The normal allele could be unable to compensate for the 50% deficiency created by the disease allele, especially when expression of both alleles is critical at a rate-limiting step in a metabolic pathway or development process. Alternatively, dimerisation of the disease protein with the normal protein could lead to a greater deficiency (>50%) at the cellular level than would be expected by the loss of one allele alone.
- A *two-hit*. The normal allele could be inactivated by a second mutation in some cells, leading to total loss of protein expression in these cells with resultant aberrant cell behaviour.

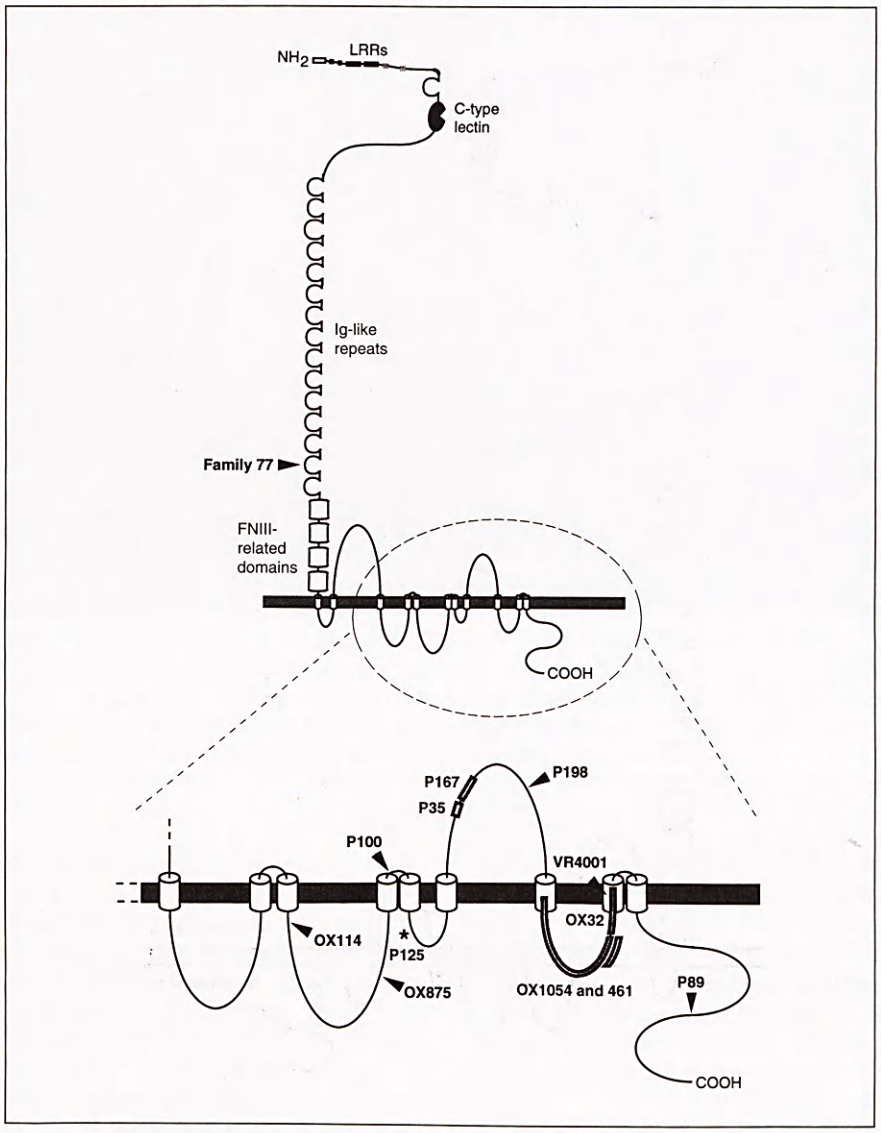

Figure 3. The position of some of the first mutations identified in the C-terminal region of the PKD-1 gene (arrows: position of frame-shifting or other large changes disrupting the protein; boxes: in-frame deletions or splicing changes; *: missense mutation; Ig: immunoglobulin; LRR: leucine-rich repeat). *Reproduced, with permission, from Ref 8.*

Which of these disease mechanisms operates in the generation of PKD-1 cysts? Current evidence favours a *loss* of function rather than a *gain* of function mechanism, either through haplo-insufficiency or by a second somatic mutation. Evidence for haplo-insufficiency has come from detailed characterisation of PKD-1 mutations, both in typical adult-onset families (as mentioned above) and in an interesting group of children with both tuberous sclerosis (TSC) and severe infantile renal cystic disease. Significantly, the severe renal phenotype in these children is almost invari-

ably associated with a complete deletion of both TSC-2 and PKD-1 genes, implying that total inactivation of one PKD-1 allele is important in the generation of renal cysts, at least in this subgroup of patients[12]. In addition, the described PKD-2 mutations are clearly inactivating[4].

Evidence has been put forward very recently to suggest that a second somatic mutation is necessary for cyst formation to occur in PKD-1. Independent work from two groups has shown that at least some cysts are clonal in origin (ie they arise from a single cell) and that a proportion of cysts examined (ca 17–24%) appear to have lost the normal PKD-1 allele[13,14]. This process is random, so could explain the focal nature of cyst formation[15] as well as the marked phenotypic variability which exists within PKD-1 families[10]. However, the relatively low proportion of cysts showing loss of the normal PKD-1 allele is a strong argument against this being a primary event. These observations also clearly conflict with previous observations indicating increased polycystin immunoreactivity in cyst epithelium and PKD-1 gene expression in PKD-1 kidneys[7].

Expression of polycystin during renal development appears to be necessary for normal tubular formation since severe cystic disease has been observed *in utero* in PKD-1[16], and expression from both alleles could be critical during this stage of nephrogenesis. Finally, it is possible that the process which confers an aberrant 'cystic' phenotype in any given cell is a chance event, affecting other factors ('modifying' factors) which determine normal PKD-1 gene expression or even other cyst-forming genes (eg PKD-2 or PKD-3) rather than a second somatic mutation leading to loss of the normal PKD-1 allele.

Future research directions and clinical implications

Perhaps the most important implication of cloning the PKD-1 gene is that the primary defect in this common disease can now be studied. We need to gain a better understanding of how polycystin functions in health and disease, the molecules with which it interacts, what factors modify the disease phenotype, and the basic molecular mechanism by which cysts arise. Answers to these questions will be essential for the development of novel therapies (eg to halt disease progression). The development of both PKD-1 transgenic and 'knock-out' animals would provide important answers as well as appropriate disease models for testing new therapies. Since PKD-1 is a systemic disease, the investigation of its function in different cell types should also be paramount.

Finally, the identification of PKD-1 will aid diagnosis. If a family carries a known mutation, it will be possible to provide an accurate genetic diagnosis for asymptomatic individuals within that family or for those in whom the diagnosis remains uncertain on clinical grounds alone. Until more efficient mutation screening methods are developed for PKD-1, however, the use of genetic linkage methods in well characterised families and renal ultrasound scanning should remain the investigations of choice.

Key Points

- The PKD-1 gene is located on chromosome 16p. It consists of 46 exons, occupies a genomic area of 52 kb and produces mRNA transcript of 14 kb
- Two-thirds of PKD-1 is duplicated more proximally on chromosome 16p. This duplicate area encodes 3 homologous genes of unknown function, with substantial homology to PKD-1
- Polycystin is a 460 kDa membrane glycoprotein expressed primarily by renal epithelial cells in the kidney
- During nephrogenesis, polycystin expression peaks in mature renal tubules, suggesting that it may play a role in the maintenance of epithelial organisation and differentiation
- There is a high rate of new mutation for PKD-1 in the population
- No clear genotype-phenotype correlation for PKD-1 has emerged so far
- Genetic linkage studies and renal ultrasound scanning remain the investigations of choice in the study of patients with suspected ADPKD

Acknowledgements

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Malaria and acute renal failure

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Of the four human malaria parasites, only *Plasmodium falciparum* causes acute renal failure (ARF), though *Plasmodium malariae* can cause a chronic nephropathy leading to nephrotic syndrome and eventual chronic renal failure. In areas of the tropical world where *P falciparum* is endemic and its transmission stable, severe malaria affects mainly children; it is manifested as either coma (cerebral malaria) or severe anaemia and claims more than a million young African lives a year. Surviving children in these populations gradually develop a degree of immunity to falciparum malaria. Adults are infected frequently by the parasite but rarely develop severe disease. Although hypoglycaemia and lactic acidosis are common findings in African children with severe malaria, multi-organ failure involving the kidneys or the liver is extremely rare. However, in areas of the world where transmission of falciparum malaria is unstable and the risk of infection low, or when non-immune individuals visit any area where falciparum malaria is endemic, severe malaria may occur at any age. In this setting, severe malaria frequently takes the form of a multi-system disorder, variably causing ARF, jaundice, coma, lactic acidosis, hypoglycaemia, anaemia, pulmonary oedema and haemodynamic shock. Hence, ARF is a common finding among cases of 'imported' severe malaria seen in hospitals in the Northern hemisphere, and its diagnosis and treatment are important components of the management of such cases.

In a recent study of 560 cases of severe adult malaria in Vietnam[1], 28% of patients had renal failure (defined as plasma creatinine ≥264 μmol/l (3 mg/dl)) on admission, and

Key Points

- ARF is a feature of severe falciparum malaria in non-immune individuals. It is commoner in adults but can occur in children
- Clinically, ARF in severe malaria takes the form of ATN, though it may be non-oliguric in the less acute form
- Volume repletion should be carried out with care, as malaria patients may develop pulmonary oedema at mildly raised or even normal filling pressures. Measurement of central pressures is mandatory
- Treatment with haemofiltration or dialysis should be early, as patients with severe malaria are hypercatabolic and non-renal causes of acidosis (lactic acidosis) frequently co-exist
- Blackwater fever, once much feared, is now rarely associated with dialysis-requiring renal failure