Cyst formation in ADPKD: new insights from natural and targeted mutants

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The 31st American Society of Nephrology meeting in Philadelphia (25–28 October 1998) provided a unique opportunity to catch up on the fast moving field of research into the pathogenesis of autosomal dominant polycystic kidney disease (ADPKD). Firstly, from the analysis of germline mutations in PKD1 and PKD2 patients, the genetic basis underlying cyst formation in ADPKD is now clear. Secondly, the consistent findings of somatic PKDJ mutations in PKD1 cyst epithelia is changing our concepts as to how cysts arise in the first place. Finally, the characterization of PKD1 and PKD2 knockout mice has led to unexpected insights into when and how cysts arise in different tissues.

The spectrum of mutations in PKD1 and PKD2

Detailed mutational analysis of the PKDJ gene has proved difficult especially in the region of the gene which is duplicated. Nevertheless, most of the duplicated region has now been analysed using methods which exploit subtle differences between the HG (homologous genes) and PKDJ sequences [1,2]. These studies have given us a better idea of the spectrum of mutations present in PKD1 families. First of all, they have revealed that the majority of mutations detected are private, indicating a high rate of new mutation. Secondly, most of the mutations are stops or frame-shifting changes which would inactivate the gene. Thirdly, no mutation 'hot spot' or genotype-phenotype correlation has been clearly identified in the region surveyed so far.

Like PKD1, the majority of germline changes in PKD2 are unique inactivating change but unlike PKD1, mutational analysis of the whole of PKD2 has been much more rapid [3]. Evidence for a mutational 'warm spot' (in proximity to a polyadenosine tract) has been proposed but like PKD1, no clear genotype-phenotype correlation has been shown, at least for renal disease severity [4]. Taken together, these results suggest that the first step leading to cyst formation in PKD1 and PKD2 involves the inactivation of one functional copy of the corresponding gene.

Susceptibility of PKD1 to new mutations

Given the prevalence of PKD1 in the population and the high rate of new mutation, a question that has arisen is why the human PKD1 gene should be so susceptible to new mutations. Preliminary evidence for two interesting hypotheses have been presented. The first proposes that a unique structural feature of PKD1 (a long polypyrimidine tract in intron 21) is what makes the gene more susceptible to mutation [5]. In theory, this tract could promote triplex DNA strands to form, leading to an increased error rate by several mechanisms. The second hypothesis proposes that gene conversion events (between the HG loci and the PKD1 gene) during recombination could lead to an increased mutation rate [6]. Each hypothesis gives rise to a logical prediction. If the first mechanism is operative, further mutational analysis should reveal a clustering of mutations around intron 21, particularly of gene deletions. If the second mechanism were true, then a high frequency of identity between PKD1 mutations and the corresponding HG sequences should be observed. Evidence for the latter should be forthcoming with a more complete analysis of the duplicated region of PKD1 and the HG loci. These mechanisms may also help to explain why there is an apparent high rate of somatic mutation at PKD1 (see below).

Somatic PKD1 mutations in PKD1 cysts

There is now a consensus that the mutational mechanism in both PKD1 and PKD2 is likely to be a 'loss of function' mechanism. However, while the presence of a germline mutation is a necessary first step, it is clearly insufficient to trigger cystic behaviour in any given susceptible cell, especially given the observed focal and sporadic nature of cyst formation. This has in turn led to the idea that an additional event: a second step, is required for cysts to form.
The nature of this additional event has been the subject of detailed study [7,8] and some debate [9]. Attractive evidence has been put forward to suggest that this trigger may involve inactivation of the normal polycystin protein by somatic mutation: a ‘two hit’ model, better known among researchers of tumour biology. Using loss of heterozygosity (LOH) analysis of PKD1 (a technique utilizing polymorphic microsatellite markers flanking the PKD1 gene), two groups found LOH involving the normal PKD1 gene in ~20% of PKD1 cysts analysed [7,8]. In addition, a somatic mutation consisting of a 2 base pair deletion inactivating the normal PKD1 gene was detected in one cyst, implying that other smaller changes inactivating PKD1 might be present in LOH negative cysts [8]. More recently, similar changes have been reported in PKD1 liver cysts [10].

Perhaps the most striking feature of these findings is that, where it was possible to distinguish between the normal and mutant PKD1 alleles, somatic mutations invariably occurred on the background of the normal allele. At first glance however, these results appear to conflict with several published studies showing prominent polycystin-1 expression in PKD1 cystic epithelia [9]. One possible explanation that may reconcile the observations of somatic mutations and persisting polycystin-1 expression is that what is being detected in cyst epithelia is inactivated or non-functional protein. This would be the case if the majority of germline and/or somatic mutations turn out to be missense. Analysis of the whole PKD1 gene in individual PKD1 cysts would help clarify the full spectrum of somatic PKD1 mutations. However, given the present difficulties in analysing PKD1 and the similarity in phenotype between the two diseases, a more feasible approach might be to examine PKD2 cysts for somatic mutations in PKD2.

**Somatic inactivation in PKD2 knockout mice**

Similar to the human disease, heterozygous PKD2 knockout mice (PKD2+/-) develop kidney and liver cysts [11]. Strikingly, mice heterozygous for an unstable PKD2 allele (which undergoes somatic inactivation by intragenic homologous recombination to form a true null allele) showed many more cysts than mice heterozygous for just a stable PKD2 null mutation. Consistent with this, cysts that develop in PKD2+/- mice showed no polycystin-2 expression by immunohistochemistry. These results contrast with our preliminary findings in human PKD2 cystic tissue [12] but the PKD2+/- mice develop relatively few cysts and further analysis may reveal that some cysts express polycystin-2, where somatic missense changes have occurred. Overall, these results nevertheless provide strong support for a ‘two hit’ model of cystogenesis in PKD2.

Whilst a cystic phenotype was not unexpected in these mutant mice, there was a surprising difference in the rate and timing of cyst formation between different organs. Like PKD1-/- mice [13], PKD2-/- mice had numerous kidney and pancreatic cysts in utero but no liver cysts [14]; liver cysts were however observed in PKD2+/+ mice with increasing age [11]. Although the full phenotype of PKD1+/+ mice has not yet been reported, these observations suggest that the function of both polycystin proteins lies more in maintaining biliary ductal organization than in their formation. They also imply that the process of cyst formation not only occurs during development but may also occur throughout adult life.

In the light of the current evidence, a ‘two hit’ model for cyst initiation seems plausible. Such a model is attractive because it could account for the focal nature of cyst formation and the phenotypic variability seen between affected individuals within the same family. However, there may be other mechanisms influencing disease severity in ADPKD, albeit less well defined. These include the effect of modifier genes and mutant polycystin proteins on expression of the disease phenotype.

**Infantile-onset PKD1 and modifier genes**

Very rarely, severely affected infants or children may be born to a mildly affected parent with PKD1. Many such cases have been described and a striking difference between these ‘early onset’ cases and the more typical adult-onset cases is both the number and the phenotype of renal cysts (mainly glomerular) found in these individuals. It is interesting to note that the increase in disease severity is confined to the kidney. These cases also resemble homozygous PKD1-/- mice, in terms of their renal phenotype although so far, there have been no reports of severely affected offspring born to two affected parents with ADPKD. Typically, the child or foetus carries the same stable mutation as the affected parent [15]. Nevertheless, a high recurrence risk (~45%) has been reported in the subsequent offspring of the affected parent, sometimes with different partners. These findings strongly suggest that other inherited factors (so called ‘modifying genes’ which may be transmitted from the affected parent) can modify the rate of cyst formation. If the ‘two hit’ model is correct, these genes might specifically alter the rate of somatic mutation at PKD1. Alternatively, these genes could encode allelic variants of other proteins which might modulate the activity of polycystin-1 by protein–protein interactions in a cystic pathway or complex.

The identification and mapping of these loci would thus provide valuable insights into the mechanism/s governing the initiation and rate of cyst formation. The influence of genetic background in suppressing or accelerating the rate of cyst formation has been convincingly demonstrated in a recessive mouse model of cystic disease (pey) and two modifying gene loci mapped [16]. The availability of PKD1 and PKD2 mutant mice now opens the possibility of other loci specifically modifying cyst formation in ADPKD being
PKD1 knockout mice: phenotypic differences between del4 and del34 mutants

Two different mouse mutants with targeted deletions of PKD1 in exon 4 (del4) and exon 34 (del34) have been described [13,20]. The full description of heterozygous mice carrying either change is incomplete but homozygous mice bearing either PKD1 mutation develop kidney and pancreatic cysts. Interestingly, del4 mice appear to have a more severe phenotype than del34 mice suggesting that a truncated polycystin-1 protein (containing all of the extracellular N-terminal region) could be functional [20]. The demonstration of such a protein is awaited but if this were the case, it could also be predicted that del4 heterozygous mice would have more cysts than del34 heterozygous mice. An alternative explanation is that these observed differences are the result of differences in genetic background between these two mutants. These observations also contrast with the absence of any clear correlation so far between disease severity and the position or nature of PKD1 mutations in man.

In summary, analysis of germ line PKD1 and PKD2 mutations has shown that a necessary first step in cyst formation in ADPKD is the loss of one functioning polycystin protein. The evidence for somatic mutation of the normal PKD1 gene in individual PKD1 cysts and for somatic mutation of PKD2 in the cysts of PKD2+/- mice suggests that loss of the corresponding normal polycystin protein may be the rate-limiting second step determining the acquisition of a cystic phenotype. These two observations provide a rationale for gene replacement as a therapeutic option. Nevertheless, the timing of such therapeutic intervention is clearly important in view of the different times at which kidney and liver cysts arise. Finally, the influence of genetic modifiers which can alter the rate of cyst formation is increasingly recognized. The identification of such genes could lead to powerful alternative therapeutic strategies to halt cyst initiation or expansion in ADPKD.

References