Multilocus polycystic disease

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The cystic diseases of the kidney are a group of diverse inherited disorders with a single feature in common: the presence of fluid-filled epithelial cysts arising from nephrons. The group includes one of the most common mendelian disorders of Caucasians, autosomal dominant polycystic kidney disease (ADPKD), as well as an array of complex developmental disorders in which renal abnormalities are but one feature. With increasing attention focused on the genomic localization of mutations that cause polycystic kidney disease, it is becoming clear that mutations at a number of distinct loci can produce renal cystic disease (Table 1). There is no shortage of theories on the molecular basis of cystic kidney disease either as was evident at a recent meeting* on renal disease.

Richard Woychik (Oak Ridge National Laboratory) presented data on a transgenic insertion (*Tg737*) in mouse chromosome 14 that leads to polycystic kidney disease in the homozygous state. The phenotype was detected as a result of an extensive screening program aimed at identifying phenotypes associated with transgenic insertions. The transgene acts as a tag for the insertion site and provides a relatively simple method of isolating the flanking DNA. Woychik and colleagues were able to identify a transcribed sequence interrupted by the transgene in *Tg737* and are in the process of characterizing the gene. There is a remarkable similarity between the phenotype of mice homozygous for the insertion and the phenotype of the common form of human autosomal recessive polycystic kidney disease (ARPKD). The distribution of the cysts within the kidney is almost identical in the human and murine diseases and the hepatic fibrosis which is characteristic of ARPKD is also found in the mouse. Linkage studies in human ARPKD have proved negative so far, but the gene disrupted in *Tg737* mice is an excellent candidate.

*'Molecular Genetics of Renal Diseases', Reston, Virginia, USA. March 28-30, 1992.

Two hits?

One of the striking features of ADPKD is the absence of a detectable abnormality in most nephrons. Even in the end-stage disease, less than 10% of the roughly 1,000,000 nephrons in each kidney contain cysts and yet every cell within the nephron carries a dominant mutation. Presumably, the mutation found in the germline is not in itself sufficient to produce a cyst and a second sporadic event, genetic or otherwise, is also required. Since any segment of the nephron (from the glomerulus to the collecting duct) may harbour a cyst, it is unlikely that either the metabolic or electrolytic gradients within the kidney are important in cyst formation. An intriguing possibility is that somatic mutation occurs in the chromosome that does not carry the inherited mutation at sites of cyst formation (S. T. Reeders, Yale University). Thus, like retinoblastoma, ADPKD could be an example of the two-hit model of pathogenesis: the disease is inherited as a dominant characteristic but knockout of both ADPKD genes is required at the somatic level to produce a cyst. In this model, each cyst represents a clonal tumour which, because some of the transport functions of its constitutent cells are preserved, is fluid-filled.

Although there is no molecular evidence to support the two-hit model and clonality of cyst wall epithelium has not as yet been demonstrated, there is considerable evidence to suggest that cyst formation is associated with focal hyperplasia of tubular epithelium. First, mice made transgenic for one of several oncogenes (SV40 T-antigen, *c-myc*) develop marked epithelial hyperplasia and polycystic kidney disease provided that the oncogene is controlled by regulatory sequences that ensure its expression in tubular epithelium[1,2]. Polycystic diseases occurs irrespective of the site of transgene insertion and the extent of cystic change correlates with the expression of the transgene. Frank solid tumours are often observed in the most severely affected animals[1]. In ADPKD, marked focal cellular hyperplasia is regularly observed in a small fraction of cyst walls but frank solid tumours are rarely observed[3]. ADPKD cannot, therefore, be considered an hereditary tumour diathesis according to classical criteria. Nevertheless, there are several diseases in which renal tumours and cysts co-exist, including hereditary tumour syndromes such as Von-Hippel Lindau syndrome and tuberous sclerosis as well as acquired cystic disease[4].

A prediction of the two-hit model is that renal cysts will occasionally be found in individuals without an inherited predisposition as a result of the acquisition of two somatic mutations by a single cell. One or two renal cysts are a common radiological finding in the general population and the probability of finding a cyst in an individual does, as predicted, rise with age. The simple two-hit model also predicts that the number of cysts should increase throughout life as second hits are gradually and progressively accumulated. Indeed, gradual enlargement of kidneys during adulthood is an almost universal feature of the disease although it is not clear whether change in the number or size of cysts is responsible for the enlargement. However, in the few cases in which the presence of an ADPKD mutation has been detected in an 11–14 week fetus by linkage analysis, dilatation of a substantial proportion of tubules was already apparent[5]. These observations do not fit the two-hit model unless the dilated tubules found in the fetus are not the progenitors of adult cysts but simply another manifestation of the mutation. In order to resolve this issue, the natural history of cyst formation needs to be studied. This is virtually impossible in man, but the recent discovery of late-onset autosomal dominant polycystic kidney disease in the rat[6] and Persian cat[7] may help.

Repeats

Is there another type of sporadic genetic event that could account for the distribution of cysts within

Table 1 Cystic diseases of the kidney

Disease/mutation	Mode of inheritance	Species	Human chromosome	Mouse chromosome	Reference
		Naturally-occurring phenotypes			
ADPKD (PKD1)	Dominant	Man	16p.13.3	17	15, 11, 12
ADPKD (non-PKD1)	Dominant	Man	?	?	16, 17
ARPKD	Recessive	Man	not 16p13.3	?	18
Medullary cystic disease	Dominant	Man	?	?	
Nephronophthisis	Recessive	Man	?	?	
von Hippel-Lindau disease	Dominant	Man	3p24-3p26	6	19
Tuberous sclerosis		Man	9q34, 11q, 12q	2 or 4, ?, ?	20, 21
cpk/cpk	Recessive	Mouse	2 or 7	12	22
cpy/cpy	Recessive	Mouse	3	9	23
bpk/bpk	Recessive	Mouse	?	?	
nm1633/nm1633	Recessive	Mouse	8p22 or 19p13.2	8	[a]
Not named	Dominant	Persian cat	?	?	7
Han:SPRD	Dominant	Rat	?	?	6
Not named	Recessive	Mink	?	?	24
		Transgenic insertional mutants			
SR2-3 (chimaeric)	Dominant	Mouse	?	11	25
Tg737/Tg737	Recessive	Mouse	?	14	[b]

[a] E. Birkenmeier (Jackson Laboratory, Bar Harbor), personal communication.
[b] R. Woychik (Oak Ridge National Laboratory) see text.
For a complete list of diseases in which renal cysts are found, see references 26 and 27.

ADPKD kidneys? An intriguing possibility is that the PKD1 gene contains an heritable unstable repeat element of the type that has been found to underlie fragile X-linked mental retardation (FMR), myotonic dystrophy (DM) and the androgen receptor abnormalities in spinal and bulbar muscular atrophy[8]. In each of these disorders there is variation in the number of trinucleotide repeats in the disease gene both in the normal population and in affected individuals, with a higher number of repeats found in affected individuals. Unstable trinucleotide repeat arrays are found in the coding region of the androgen receptor, leading to spinal and bulbar muscular atrophy, and in the untranslated regions of the DM and FMR1 genes. Although the mechanism of the effect of a particular number of repeats on the functioning of the genes in these disorders is not understood, there is a good correlation between repeat number and phenotype.

One of the features of FMR, DM, Huntington's disease and a number of other genetic disorders, is anticipation — an increase in the severity of the disease in successive generations. In FMR, for example, normal males have about 6–60 copies of a CCG repeat in the 5' untranslated region of the FMR1 gene, whereas carrier males have 60–200 copies and mentally retarded individuals have more than 200(ref. 8). In the context of ADPKD, the germ-line abnormality might simply be a locus-encoded predisposition to further instability at the same locus. Repeat number instability at the somatic level might explain the apparently sporadic distribution of cysts within the kidneys. Indeed, somatic mosaicism of trinucleotide repeat number is a prominent feature of the DM and FMR1 genes in affected individuals. Classical anticipation, a stepwise progression in severity in successive generations, does not occur in ADPKD. Nevertheless, germ-line repeat instability might account for the rare cases in which massive renal involvement and death in the neonatal period occurs in the offspring of parents who have the typical adult-onset form of the disease[9,10].

A series of genomic clones have been isolated[11] spanning the entire PKD1 region defined by flanking genetic markers[12]. At least 23 genes, containing >65 kilobases (kb) of transcribed sequence, have been found in the region (our unpublished data), but screening for mutations will present a formidable task. The attraction of the unstable repeat hypothesis is that it can be rapidly tested using the genomic clones for the PKD1 region.

Polarity

In normal adult kidneys, the sodium pump (Na^+-K^+-ATPase) is active in the basolateral membrane of the polarized epithelium where it is largely responsible for vectorial transport of sodium from the tubule lumen into the blood. In ADPKD kidneys, Na^+-K^+-ATPase is localized to the apical membranes of cells in the epithelial lining of cysts but it is found in the basolateral membranes of cells from normal tubules in the same kidney[13]. Polarization abnormalities have previously been observed in renal epithelium after ischemic injury but in ischemic epithelium Na^+-K^+-ATPase is found on both sides of the cell so that it is unlikely that the changes in ADPKD are a consequence of ischemia. Patricia Wilson (Johns Hopkins University Medical School) provided additional data on polarization abnormalities in ADPKD. Several other basolateral proteins, including EGF receptor, ankyrin, fodrin and uvomorulin, are mislocated to the apical surface indicating that the reversal of polarity is not specific to Na^+-K^+-ATPase. Nor is the phenomenon confined to

basolateral proteins because the apical Na^+-K^+-$2Cl^-$ transporter is mislocated to the basolateral membrane in cystic epithelium. Moreover, several proteins, including the band 3 anion transporter, gp330 antigen, laminin and type IV collagen are located normally in cystic epithelia.

Further evidence for a polarization defect in renal cystic disease was reported by Ellis Avner (University of Washington) who studied the distribution of Na^+-K^+-ATPase in *cpk/cpk* mouse kidneys. Na^+-K^+-ATPase was found on basolateral surfaces in both normal and cystic proximal tubules at all stages of development. In normal mice, the pump is located on both cell surfaces of collecting tubules at birth but found predominantly on the basolateral membrane by the 12th postnatal day. In *cpk/cpk* mouse kidneys, cysts develop in collecting ducts during early postnatal life. At the 12th postnatal day, antibodies to the α and β subunits of Na^+-K^+-ATPase react with both apical and basolateral surfaces of the cystic collecting ducts in these animals. These data suggest that a developmental event which is responsible for determining the mature pattern of membrane protein is absent or delayed in the collecting ducts but not the proximal tubules of *cpk* homozygotes.

The normal mechanism for regulation of the distribution of Na^+-K^+-ATPase in renal epithelia has been elegantly studied using cultured Madin-Darby canine kidney (MDCK) cells as a model[14]. After cell–cell contact is induced in MDCK cultures there is a change in distribution of Na^+-K^+-ATPase staining such that >85% is found at the basolateral cell surface after 96 hours. Vectorial delivery of the enzyme is the same for both surfaces but the residence time was much longer for basolateral protein. Hammerton *et al.*[14] suggest that Na^+-K^+-ATPase is stabilized at the basolateral surface by binding to a membrane–cytoskeletal complex which is lacking at the apical surface. This suggests several sites for the defect in polycystic kidney disease:

1. A component of the cytoskeletal complex is abnormally distributed.
2. The endocytic mechanism that is normally responsible for turnover of apical membrane components that are not bound to cytoskeletal matrix is inactive or inefficient.
3. One of the signals that direct polarization following cell–cell contact is missing.

Wilson[13] has suggested that reversal of polarization may be the proximate cause of renal cyst formation in ADPKD. Ouabain-sensitive vectorial transport of sodium ions was found to be reversed in confluent ADPKD epithelia *in vitro*. It remains to be seen whether secretion of sodium into the tubular lumen is the net result of polarization abnormalities *in vivo*. In *cpk* mice it is unlikely that abnormalities in Na^+-K^+-ATPase transport are responsible for cyst formation as cystic proximal tubules do not show these changes. □

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Fragile X founder effect?

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The genetics of fragile X syndrome — the most common form of inherited (X-linked) mental retardation — are enigmatic: fragile X pedigrees may contain normal transmitting males and the risk of mental impairment depends on the individual's position within the pedigree[1]. Recent progress has included the cloning of the putative gene[2,3], *FMR-1*, and the demonstration that the mutation involves the amplification of the repetitive sequence $(CCG)_n$[4,5] located 5' to *FMR-1*. An increase in the number of repeats in successive generations accounts for the observed increasing penetrance (anticipation). Nevertheless, a fundamental paradox remains unresolved. Because fragile X mutations are constantly lost from the population, the high frequency of the mutant allele(s) must be due to new mutations that constantly arise. Segregation analysis has estimated this mutation rate at 7.2×10^{-4} in sperm, implying that >50% of random carrier females are new mutations[1], yet no fresh mutations have been detected. A possible resolution to this dilemma is reported by Richards *et al.*[6] in this issue: haplotype analysis of fragile X chromosomes reveals that a few founder (ancestral) mutations are responsible for most fragile X cases. Moreover, the haplotypes on which the mutations occurred may be particularly susceptible to amplification of the $(CCG)_n$ repeat to the full fragile X mutation. ▶