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PERSPECTIVES IN BASIC SCIENCE

Molecular pathogenesis of ADPKD: The polycystin complex gets complex

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Molecular pathogenesis of ADPKD: The polycystin complex gets complex. Autosomal-dominant polycystic kidney disease (ADPKD) is one of the most common human monogenic diseases with an incidence of 1:400 to 1:1000. It is characterized by the progressive development and enlargement of focal cysts in both kidneys, typically resulting in end-stage renal disease (ESRD) by the fifth decade. The cystogenic process is highly complex with a cellular phenotype consistent with "dedifferentiation" (i.e., a high proliferative rate, increased apoptosis, altered protein sorting, changed secretory characteristics, and disorganization of the extracellular matrix). Although cystic renal disease is the major cause of morbidity, the occurrence of nonrenal cysts, most notably in the liver (occasionally resulting in clinically significant polycystic liver disease) and the increased prevalence of other abnormalities including intracranial aneurysms, indicate that ADPKD is a systemic disorder. Following the identification of the first ADPKD gene, *PKD1*, 10 years ago and *PKD2* 2 years later, considerable progress has been made in defining the etiology and understanding the pathogenesis of this disorder, knowledge that is now leading to the development of several promising new therapies. The purpose of this review is to summarize our current state of knowledge as to the structure and function of the *PKD1* and *PKD2* proteins, polycystin-1 and -2, respectively, and explore how mutation at these loci results in the spectrum of changes seen in ADPKD.

AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) IS GENETICALLY HETEROGENEOUS

Mutation to *PKD1* (chromosome region 16p13.3) is the most common cause of ADPKD (~ 86% cases) with most of the remainder due to changes to *PKD2* (4q22). However, the description of possible unlinked families indicates that at least one other unknown gene may be associated with ADPKD. The clinical phenotypes of PKD1 and PKD2 are closely related; they were only recognized as diseases with different etiologies by genetic linkage analysis in the late 1980s. This phenotypic similarity includes both the nature of the renal disease and the range of extrarenal manifestations (cases of polycystic liver disease and intracranial aneurysms are associated with both genes). However, there are differences: PKD2 is a significantly milder disease in terms of the mean age at diagnosis, a lower prevalence of hypertension, and a later age at onset of end-stage renal disease (ESRD) (PKD1, 54.3 years and PKD2, 74.0 years) [1]. Furthermore, while on average disease severity is similar between males and females in PKD1, PKD2 females have a significantly better prognosis (age at onset of ESRD in males, 68.1 years and in females, 76.0 years). The reason for this difference is unclear [2, 3]. Within both disorders, there is a wide range of intrafamilial phenotypic variability, seen both in terms of the severity of renal disease and manifestations of extrarenal abnormalities, indicating that genetic modifying loci and environment factors significantly influence the course of the disease [2].

THE MUTATION SPECTRUM

PKD1 is a complex gene with 46 exons that generates a large transcript (~14 kb) containing a long open-reading frame predicted to encode a 4302aa protein. Characterization of the gene structure and identification of mutations has been complicated by genomic duplication of the 5′ region of *PKD1* (to exon 33) such that approximately six copies of *PKD1*-like genes, with various rearrangements relative to *PKD1*, are located in 16p13.1. Sequence similarity of these pseudogenes (many of which encode transcripts but probably not significant protein products) to *PKD1* means that particular anchored, long and locus-specific amplification methods are required to characterize and screen *PKD1* for mutations [4]. *PKD2* has 15 exons, generates a ~5 kb transcript, and encodes a protein of 968aa.

Both *PKD1* and *PKD2* exhibit marked allelic heterogeneity, with approximately 200 different *PKD1* and over

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50 different *PKD2* mutations described [3–5]. The majority of these are unique to a single family, illustrating the complexity of gene-based diagnostics for these disorders and indicating that a significant level of new mutation is occurring [4]. Most mutations are predicted to truncate the protein (due to frame-shifting deletion/insertion, nonsense changes, or splicing defects), but a significant level of in-frame and missense changes have also been described [3, 4]. Mutations are found throughout both genes, although more *PKD1* changes are found in the 3′ half of the gene [4]. This pattern of mutation is consistent with inactivation of one allele but recent genotype/phenotype correlations in *PKD1* suggest that not all mutations may have the same phenotypic outcome. In *PKD1*, mutations 5′ to the median are associated with more severe disease (average age at onset of ESRD at 5′, 53 years and at 3′, 56 years) and a significantly greater risk of developing intracranial aneurysms [2, 5]. This association is not related to mutation type and may be due to the proposed cleavage of polycystin-1 into two different proteins (see later), with mutations to each half having potentially different phenotypic consequences [5, 6]. As yet, no clear phenotype/genotype correlations have been reported for PKD2 [3].

MUTATIONAL MECHANISM

A two-hit mechanism of cyst formation has been proposed for ADPKD (consisting of a germ line mutation to one allele and a somatic mutation to the other). This is an attractive hypothesis which could help explain both the focal nature of cyst development and the striking phenotypic variability seen in most families. Evidence of epithelial cell clonality within individual cysts and the detection of somatic mutations in cells isolated from a proportion of renal and hepatic cysts supports the two-hit hypothesis [7–9]. Furthermore, embryonic renal cyst development in homozygous knockout *Pkd1* or *Pkd2* animals, and particularly the progressive cystic disease seen associated with the *Pkd2*^{WS25} mutant (that has a *Pkd2* allele prone to inactivation by somatic mutation), are consistent with a two-hit model of cyst development [10, 11]. However, there remain questions as to whether the two-hit mechanism is the only means to generate a cyst and indeed, whether these somatic events may be later events more important for cyst expansion and progression rather than initiation [12].

Persistent or even enhanced immunoreactivity for polycystin-1 or -2 is often detected in cystic epithelia but it is not clear if this signal represents functional protein [13–16]. Recent studies indicate that multiple karyotypic changes resulting in chromosomal imbalances are associated with cyst development, not just loss of heterozygosity (LOH) at the normal ADPKD allele (although LOH at 16p was found at a higher frequency) [17]. Progressive dedifferentiation of cystic epithelia during cyst enlargement in the *Pkd2*$^{WS25/-}$ model also indicates that cyst development and expansion may be dynamic and the result of more than a simple two-hit process [18]. A situation that illustrates the complexity of cyst development is the finding of somatic *PKD2* mutations in PKD1 cystic epithelia and vice versa, suggesting that cells that are transheterozygous may be prone to cyst development [19, 20]. However, it is apparent that this pattern of mutation cannot fully explain cyst initiation as patients and mice that are trans-heterozygous for a germ line *PKD1* and *PKD2* mutation have been described [21, 22]. Although these patients/animals have more severe disease than cases with either mutation alone, the difference is not dramatic (i.e., not every renal tubular cell gives rise to a cyst). These somatic events to the other ADPKD gene therefore appear to be akin to modifying genetic changes that increase the risk of cyst development (or hasten their progression) rather than cyst initiating events. Another example where a mutation to a second gene (in the germ line) can hasten cyst development and expansion are cases in which both *PKD1* and a tuberous sclerosis gene (*TSC2*) allele are mutated due to a large contiguous gene deletion [23]. These patients have TSC and severe early-onset PKD, indicating a likely synergistic role for polycystin-1 and tuberin (the TSC2 protein) in cyst development [24]. A possible mechanism which could account for this phenotype is the finding that tuberin plays a role in trafficking polycystin-1 to the lateral cell membrane [25].

When considering the mutational mechanism, it is worth noting that loss of a single allele (haploinsufficiency) may be sufficient in itself to elicit a phenotypic change. Recent studies have shown that *Pkd2*$^{+/-}$ vascular smooth muscle cells express a lower level of polycystin-2 and have altered intracellular Ca^{2+} homeostasis [26]. In addition, haploinsufficient *Pkd2* mice have reduced survival (not due to renal failure) indicating that a dosage reduction of polycystin-2 itself exerts a phenotypic load [27]. This is, however, not the case with Pkd1 heterozygotes who have a normal life span. Overexpression of functional polycystin-1 via a genomic transgene leads to age-related cysts in the kidney and liver, suggesting that an imbalance in the relative amounts of one polycystin protein can also result in cyst development. One possible explanation is that some polycystin complexes become inactivated due to the stoichiometric imbalance between the two proteins [28]. Given the available data, it seems reasonable to conclude that cystogenesis is a complex process. Cyst development requires a germ line mutation but beyond this, the likelihood of cyst formation is influenced by a number of different factors. These include somatic genetic events at the other (normal) allele, mutations at the other ADPKD gene and possibly a wide array of other genetic loci. In effect, these loci act as