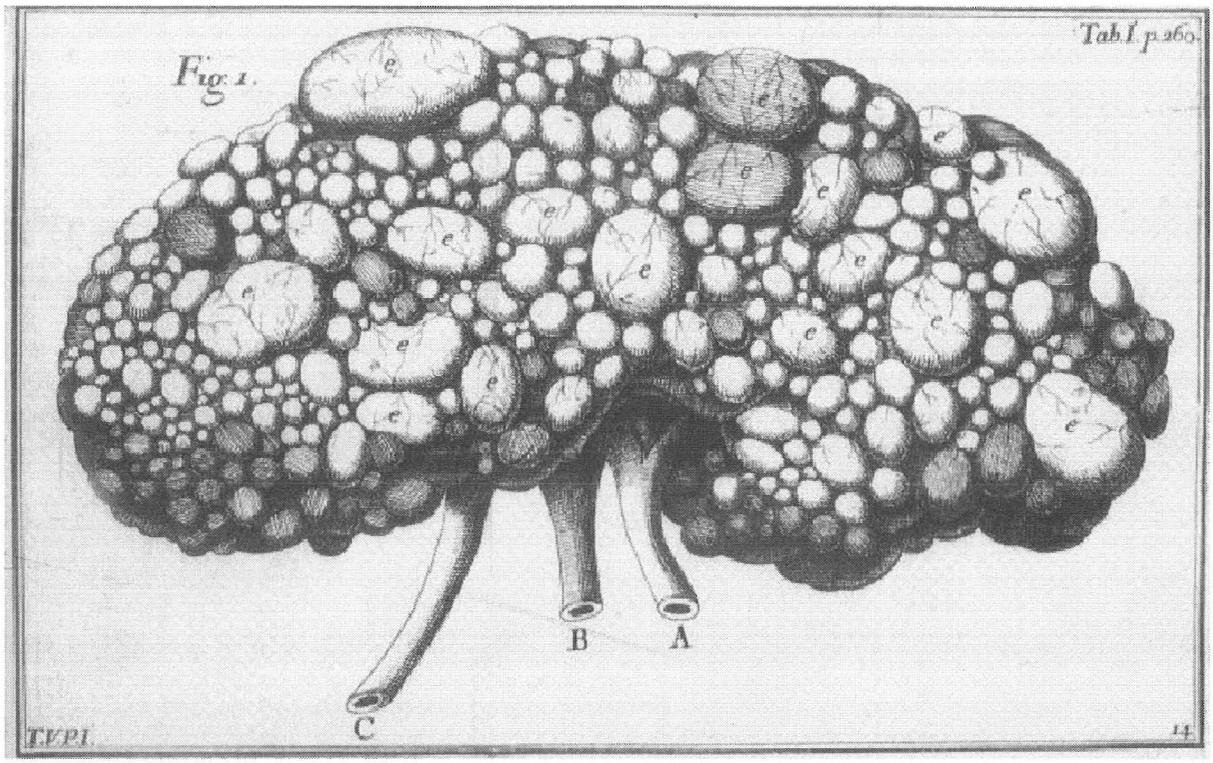
Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Peter Igarashi, M.D.

July 26, 2001



Domenico Gusmano Galeazzi, 1757

Dr. Peter Igarashi
Professor
Division of Nephrology

Dr. Igarashi is interested in kidney development, kidney-specific gene expression and polycystic kidney disease.

This is to acknowledge that Peter Igarashi, M.D., has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

Case Presentation

A 48 year-old man with autosomal dominant polycystic kidney disease (ADPKD), hypertension, and bipolar disorder presented to the Yale-New Haven Hospital emergency department in October 1994 with an exacerbation of chronic abdominal pain. Polycystic kidneys were discovered incidentally 16 years earlier during an evaluation after an automobile accident. He reported a history of episodic gross hematuria (none in the past year), no history of renal calculi, and a cyst infection that resolved with oral antibiotics 15 months ago. He has had progressive renal insufficiency, and a 24 hour urine collection one year ago showed a creatinine clearance of 17 ml/min. Over the past 4-5 months, there has been an increase in right-sided abdominal pain. He denied fever, dysuria, and gross hematuria. There were no uremic symptoms.

Past medical history was remarkable for ADPKD, bipolar disorder with frequent hospitalizations, hypertension for 5 years, multiple fractures from automobile accidents, and umbilical herniorrhaphy in 1992. There was no known family history of kidney disease. His father had a history of psychiatric illness and died 5 months after the patient's birth. Medications included carbamazepine 300 mg bid, nifedipine XL 30 mg qd, furosemide 40 mg qd, and calcium carbonate 500 mg tid with meals.

Physical examination revealed an alert, thin male in moderate distress. The blood pressure was 140/90, pulse was 84, and he was afebrile. A tender mass was palpated in the right mid-abdominal region. There was no peripheral edema.

Laboratory examination revealed a white cell count of 5,800 (71% PMNs, no bands) and hematocrit of 32. The serum electrolytes were sodium 139, potassium 4.1, chloride 111, bicarbonate 20, BUN 73, and Cr 6.5. Urinalysis showed pH 5, 1+ protein, 2-3 white cells, and 3-5 red cells. Urine culture showed no growth. A renal ultrasound revealed that the right kidney was 21.7 cm in length with a large (4.2 × 4.7 × 4.1 cm) cyst containing echogenic material. The left kidney was 20 cm.

A surgical procedure was performed.

Classification

This man presents with typical features of polycystic kidney disease, a common genetic cause of renal failure (1). Polycystic kidney disease (PKD) is characterized by the replacement of renal parenchyma with large, fluid-filled cysts. Although polycystic kidney disease was previously classified by age of onset into adult and infantile forms, more recent genetic studies have shown that this classification is not meaningful, since mutations of the same gene can cause disease arising at different ages. Accordingly, a classification based on the pattern of inheritance is now preferred. There are two patterns of inheritance that are recognized: autosomal dominant (ADPKD) and autosomal recessive (ARPKD). ADPKD is common and occurs in both children and adults, whereas ARPKD is rare and occurs primarily in children. Two disease genes that cause ADPKD have been identified. The disease gene responsible for ARPKD has been mapped to

chromosome 6 but has not yet been cloned. PKD also occurs in association with other genetic diseases, including von Hippel-Lindau disease and tuberous sclerosis (2). Tuberous sclerosis is associated with very early-onset PKD and will be discussed in greater detail below.

ADPKD is transmitted as an autosomal dominant trait with complete penetrance. The disease is genetically heterogeneous and can arise from mutations in at least two genes, PKD1 and PKD2 (3). Mutations of PKD1 located on chromosome 16p13 are responsible for 85% of cases, whereas mutations of PKD2 on chromosome 4q22 are responsible for 15% of cases. Some PKD families have been identified in which disease is not linked to either chromosome 16 or chromosome 4. This has raised the possibility that there is a third gene locus, PKD3, which has not yet been cloned. York Pei at the University of Toronto has recently shown that some individuals that were thought to have disease linked to PKD3 actually had inherited mutations of both the PKD1 and PKD2 genes. Such individuals appeared to have more severe disease than individuals with mutations of either PKD1 or PKD2 alone (4).

ADPKD

- Autosomal dominant inheritance
- Genetically heterogeneous
  - PKD1 (16p13.3)
  - PKD2 (4q21-23)
- Affects 1 in 400 to 1 in 1000 live births (>500,000 in US population; 4-6% ESRD)
- Affects all ethnic groups worldwide
- No family history in 40% of cases

Incidence

Autosomal dominant polycystic kidney disease (ADPKD) is a common disease that affects all ethnic groups worldwide (1). Studies in Western countries have shown a frequency of ADPKD ranging from 1/261 to 1/1,019 (mean 1/503). A study in Hong Kong Chinese showed a disease frequency of 1/339 (5). Thus, ADPKD is more common than many well-known genetic diseases such as cystic fibrosis (1/2,000 Caucasians), Tay-Sachs disease (1/6,000 Ashkenazi Jews), phenylketonuria (1/25,000 Northern Europeans), and Huntington's disease (1/20,000). The incidence of ADPKD is comparable to the incidence of sickle-cell disease in American Blacks. Since one-half of individuals who inherit the disease gene will progress to end-stage renal disease, ADPKD is the most common, life-threatening genetic disease in man. ADPKD may not be as well known as some other genetic diseases because it usually does not become manifest until middle age. The patient presented earlier had no family history of renal disease. Approximately 40% of affected individuals do not have a positive family history and presumably reflect new mutations. In the U.S. there are more than 500,000 individuals with ADPKD (5 million worldwide). ADPKD is the most common genetic cause of end-stage renal disease and is responsible for 4-6% of patients requiring chronic renal replacement therapy in the U.S.

History

Polycystic kidney disease has probably existed since antiquity (6). The disease gene may be more easily transmitted, since affected individuals generally do not become

ill until after their peak reproductive years. In Europe in the Middle Ages, there were frequent references to cystic disease affecting the abdominal cavity. However, it is impossible to determine how many of these cases represented polycystic kidney disease rather than hydatid cysts, which were also prevalent at the time. The first known case of PKD, at least in the West, was probably King Stefan Bathory of Poland. Bathory was born in 1533 in Transylvania and was anointed King in 1576. Although he was one of the most successful monarchs in Polish history, he is perhaps best known as the uncle of Elizabeth Bathory, upon whose life the legend of Dracula is thought to be based. King Bathory became chronically ill in the early 1580's, around the age of 50. In 1585, he experienced an episode of acute chest pain, fatigue, weakness, and loss of consciousness. It was also noted that his facial muscles twitched uncontrollably and that he had a very pale facial color. His heartbeat was weak and uneven, and he died in 1588 at the age of 53. An autopsy performed by Jan Zigulitz revealed that the kidneys were "large like those of a bull, with an uneven and bumpy surface, nothing like [we] had ever seen." Bathory's symptoms resemble those of uremia, and a review conducted in 1933 concluded that he probably died from polycystic kidney disease. By the 18th Century, PKD was recognized as an anatomic entity distinct from hydatid cysts. The cover page shows a plate created by Domenico Gusmano Galeazzi in 1757. Galeazzi was an anatomist and physician who encountered three patients with abdominal symptoms that were thought to be due to renal colic or tumors. Post-mortem examination revealed instead that all patients had enlarged kidneys, "the structure of which was entirely subverted by numerous vesicles of different sizes" (7). By the 19th Century, the clinical features of PKD had been recognized.

Clinical

Clinical manifestations of ADPKD include abdominal mass, chronic flank and/or back pain, gross hematuria, and symptoms attributable to urinary tract infection or renal colic. Approximately 45% of individuals with ADPKD will develop end-stage renal disease by the age of 60. Patients typically present in the third and fourth decade, and ESRD commences within 10 years. By ultrasound, renal cysts can be detected in virtually all patients who are heterozygous for PKD1 mutations by the age of 30. Thus, the disease is fully penetrant. However, ADPKD has variable expressivity. Only 45% of heterozygous individuals will progress to ESRD. Within a given family, the age of onset of the disease is also variable. Mutations of PKD1 and PKD2 cause identical clinical manifestations. However, the disease due to PKD2 is less severe and later in onset (mean age at ESRD of 69 versus 53) (8). In addition to causing renal failure, renal cysts can be complicated by hemorrhage, infection, urinary tract obstruction, nephrolithiasis, and intractable pain. Several of these complications were observed in the patient presented above. Hypertension is also very common in ADPKD, occurring in more than 75% of patients (9). Increased blood

Clinical features and complications

- Acute and chronic pain
  - –cyst expansion/rupture
  - –cyst hemorrhage with or without hematuria
  - –cyst/urinary tract infection
  - –nephrolithiasis (~20%)
- Hypertension (~80%)
- Progressive renal insufficiency (ESRD in 45%)

pressure has been attributed to activation of the renin-angiotensin system by renal ischemia due to cyst enlargement. More recently, it has been found that the PKD1 and PKD2 gene products are expressed in vascular smooth muscle, raising the possibility that hypertension arises from a primary defect in the blood vessels (10).

Although ADPKD is characterized by kidney cysts and renal failure, it should be regarded as a systemic disease. The PKD1 and PKD2 gene products are widely expressed in the body, and mutations of the genes can affect a number of extrarenal tissues (11). In addition to the kidney, cysts can arise in several other epithelial organs, including the liver (common), pancreas (rare), ovaries, and choroid plexus. Liver cysts are common (75% of patients) and originate from the biliary tree. Although liver cysts can become infected or bleed, liver failure does not occur in ADPKD (in contrast to ARPKD). Other extrarenal manifestations include large vessel aneurysms, cerebral dolichoectasis (a fusiform dilatation of the cerebral arteries), and colonic diverticuli. Cardiac findings include valvular abnormalities such as mitral valve prolapse, mitral regurgitation, aortic insufficiency, and tricuspid regurgitation (12). Interestingly, Pkd2 knockout mice also exhibit cardiac abnormalities, suggesting that the Pkd2 gene is required for normal heart development. Left ventricular hypertrophy is common and has been observed in normotensive individuals (13).

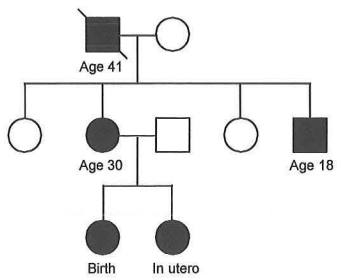
Systemic manifestations of ADPKD

- Cysts in kidney (100%), liver (75%), pancreas, ovary, choroid plexus
- Cardiac valvular abnormalities
  - Mitral valve prolapse
  - TR, AI, etc.
- Intracranial aneurysms•5% overall
  - 20% with family history
- Large vessel aneurysms
- Colonic diverticuli

Ruptured aneurysms represent the most feared complication of ADPKD (14, 15). The aneurysms primarily involve the cerebral arteries and aorta, and tend to occur in families. The mortality of ruptured aneurysms is 50%. The incidence of aneurysms is 5% in the ADPKD population overall, but 25% if there is a positive family history. Recently, both PKD1 and PKD2 gene products have been identified in the smooth muscle of elastic arteries, consistent with disease in this site. The utility of screening for asymptomatic cerebral aneurysms in ADPKD is controversial, as it is for the population at large (16). The rate of progression appears to be low (17), and the incidence of new aneurysms is about 2% annually. In contrast, patients with symptoms or prior history of subarachnoid hemorrhage should receive regular screening and/or intervention. The risk of surgery is around 10%.

Fig. 1

Autosomal dominant inheritance



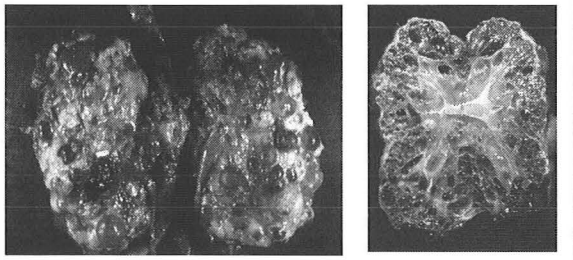
A striking feature of ADPKD is the variability of the phenotype. ADPKD is fully penetrant, meaning that virtually 100% of individuals who inherit a mutated PKD gene will develop renal cysts.

However, the severity of the disease, age of onset, and the spectrum of extrarenal manifestations vary widely between affected individuals, even within the same family. As previously mentioned, only about one-half of affected individuals will progress to ESRD. Therefore, the expressivity of the disease is highly variable. Fig. 1 shows a pedigree from a study by Patricia Gabow at the University of Colorado (18). The closed symbols indicate affected individuals. As can be seen, there is vertical transmission of disease and both sexes are affected, consistent with autosomal dominant inheritance. However, the age of onset of disease, indicated below each symbol, varies widely, even within the same kindred. That is, individuals who inherit the same gene mutation can have widely varying disease severity. Possible explanations for variable expressivity are discussed below.

Pathology

ADPKD is characterized by massive replacement of renal parenchyma with fluid-filled cysts. The cysts originate from the epithelium of the tubular nephron and occur in both the cortex and medulla. Cysts can arise from all segments of the nephron as well as the renal collecting system. Fig. 2 shows the gross pathology of the kidney from patients with ADPKD. The parenchyma is entirely replaced with fluid-filled cysts, some of which contain blood. Although cysts arise in only 1% of nephrons, they can grow to considerable size and compromise overall renal function by compressing neighboring normal nephrons. Histologically, the cysts are lined by a single layer of columnar or squamous epithelial cells.

Fig. 2 Gross pathology

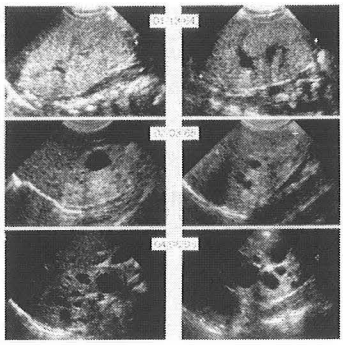


Diagnosis

Since ADPKD is an autosomal dominant trait, the probability that an individual will inherit the disease from an affected parent is 50%. The diagnosis of ADPKD in individuals at risk for disease is usually established by renal ultrasonography. The sensitivity of renal ultrasound for diagnosing renal cysts varies with age. Cyst formation in ADPKD is age-related. Cysts are less common in children but are invariably present by the age of 60. Fig. 3 shows serial renal ultrasounds of a patient with ADPKD obtained over a nine-year period. Note that multiple echolucent cysts are present in both kidneys and that the number of cysts increases over time. It is also important to remember that simple renal cysts, which are not due to ADPKD, are common in the general population, and their incidence also increases with age. Simple renal cysts are rare in children (0.2% prevalence) but are present in more than half of the population over the age of 50. Based on these considerations, Ravine et al have proposed the following ultrasound criteria for

diagnosis of ADPKD in at-risk individuals: presence of at least two renal cysts (unilateral or bilateral) in individuals younger than 30 years; presence of at least two cysts in each kidney in those aged 30-59; and four cysts in each kidney in those aged 60 years and above (19). The sensitivity and specificity of these diagnostic criteria have recently been evaluated in patients with ADPKD types 1 and 2 and compared with genetic linkage analysis (20). The sensitivity of ultrasonography in individuals younger than 30 years was 95% for PKD1 but only 67% for PKD2 (93% overall). The sensitivity in individuals aged 30 years or older was 100% for both PKD1 and PKD2. The specificity of the test was 100% in all age groups. Although the use of Ravine's criteria will lead to false-negative results in some children, these are more acceptable than false-positive results because of the anxiety produced by the diagnosis of the disease.

Fig. 3 Diagnosis of ADPKD by renal ultrasonography



Age (yrs.)	Renal cysts (no.)
0-29	2
30-59	2 in each kidney
>60	4 in each kidney

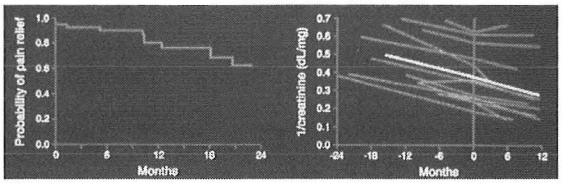
The diagnosis of ADPKD can also be established by computed tomography or magnetic resonance imaging. Prenatal genetic testing for PKD1 was first performed by Stephen Reeders from a chorionic biopsy. Linkage analysis can be performed using closely linked, polymorphic markers. Direct gene-based mutation testing of both the PKD1 and PKD2 genes is available commercially.

Treatment

There is no specific treatment for ADPKD, although many approaches have been considered including dietary modification, taxol, etc.(21). Patients who develop renal failure are managed conservatively, as exemplified by the patient presented above. The Modification of Diet in Renal Disease Study (MDRD) was a large, multicenter, prospective study to investigate the effects of low-protein diet and blood pressure control on progression of chronic renal failure. About 500 subjects with ADPKD were included in the MDRD study. A separate analysis of these patients showed that dietary protein restriction did not affect the course of disease (22). Aggressive control of BP was also not shown to be beneficial compared with standard BP control. It is important to note, however, that this study was an intention-to-treat analysis and that the actual differences in mean arterial blood pressure achieved between the groups were only 6.3 to 3.9 torr. Because hypertension is common in ADPKD, and because elevated blood pressure accelerates the progression of other forms of chronic renal disease, most clinicians believe that tight control of blood pressure is beneficial in ADPKD patients. However, there is no consensus on the optimal antihypertensive regimen. Since activation of the renin-angiotensin system has been observed in PKD, the group at the University of

Colorado has advocated the use of angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors are widely used to retard the progression of chronic renal diseases such as diabetic nephropathy but were not shown to retard the progression of established ADPKD in the Angiotensin converting enzyme Inhibition in Progressive Renal Insufficiency (AIPRI) trial (23). In contrast, a recent prospective, nonrandomized study from the University of Colorado compared ACE inhibition to diuretics and found that the decrement in creatinine clearance after a 5-year follow-up was significantly larger in the diuretic group (5.6 vs. 2.5 ml/min/1.73 m^2/year), despite similar blood pressure control (24). The National Institutes of Diabetes, Digestive and Renal Diseases (NIDDK) is currently sponsoring a prospective, randomized, multicenter study to examine whether inhibition of the renin-angiotensin system affects the progression of ADPKD.

Fig. 4 Cyst fenestration



62% of patients remained pain-free after 2 years (left panel). There was no change in the progression of renal failure (right panel).

The patient presented above developed a common complication of ADPKD, which was intractable pain from renal cysts. Although the cause of painful cysts is not known, pain correlates with size and is frequently observed in cysts greater than 3 cm in diameter. The treatment of painful renal cysts has been problematic because of the high recurrence rate. Open decortication of renal cysts was first performed by Rovsing in 1911. However, the procedure was abandoned in the 1950's after reports that it contributed to worsening renal function. Alternative approaches such as percutaneous cyst aspiration and instillation of ethanol, tetracycline, or other sclerosing agents have been performed. In the 1970's there was renewed interest in the use of surgical decompression. Fig. 4 shows results of a study from Mayo Clinic showing that open decortication often resulted in immediate reduction in pain (25). After 2 years follow-up, 62% of patients remained pain-free. Importantly, a plot of 1/creatinine revealed that the procedure did not affect the progression of renal failure either positively or negatively. The patient presented above underwent a Rovsing procedure, which relieved his pain.

More recently, laparoscopic decortication has been performed in patients with ADPKD (26). Although no direct comparisons of open and laparoscopic decortication have been reported, the results appear to be similar. Moreover, the laparoscopic procedure offers the advantages of shorter hospital stay, lower requirement for post-operative analgesia, and more rapid convalescence. The first published reports of laparoscopic decortication appeared in 1996, and several series have now been reported (27). At UT Southwestern, laparoscopic decortication is performed by Dr. Jeff Caddedu in the Dept. of Urology Center for Minimally Invasive Surgery. His approach has recently been published (28). Five cases have been performed in the past two years. In all

cases, the operation was performed on the kidney with the most pain. Decortication of more than 100 cysts was performed in each operation. Every cyst that could be visualized was decorticated, and intraoperative ultrasonography was performed to visualize cysts that were located below the surface. The patients have been followed for 6 months to 2 years, and all remain off narcotics (personal communication, Jeff Caddedu).

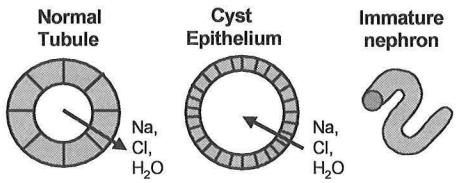
Pathogenesis

The pathogenesis of polycystic kidney disease remains unknown but has been the subject of intense investigation. As early as 1869, Virchow proposed that PKD was due to obstruction of the nephrons by uric acid crystals. By 1900, PKD was thought to be a congenital abnormality due to failure of the nephrons to connect with the collecting system. Continued glomerular filtration would then result in distension of the tubular nephron. This notion was disproved in the early 1900's when microdissection studies revealed that cysts communicate with the nephron and originate from all segments of the nephron as well as the renal collecting system.

Renal cysts are lined by a single layer of epithelium that differs from normal tubular epithelium. The cyst epithelium has a higher rate of cellular proliferation and is less differentiated than normal tubular epithelium (29). In many respects, the cyst epithelium resembles the immature epithelium that is normally present in the developing fetal kidney. Thus, there is considerable interest in our laboratory and others in studying the molecular mechanisms of kidney development since they may provide insights into the mechanisms of cystogenesis. For example, cyst epithelium is characterized by high expression of genes such as c-myc, clusterin (SGP-2), Pax-2, and Cux-1/CDP, which are normally expressed during fetal kidney development but are not normally expressed in mature tubules (Fig. 5) (30). Conversely, expression of differentiation markers such as HNF-1 and E-cadherin is inhibited in cyst epithelium. These results led Jared Grantham to propose the so-called "maturation arrest hypothesis" of renal cystogenesis (31). According to Grantham, renal cysts can arise from arrested differentiation of tubular epithelial cells, producing congenital forms of PKD. ADPKD in adults may arise from "dedifferentiation" of previously terminally differentiated tubular epithelial cells.

Fig. 5

Abnormalities in renal cyst epithelium



Proliferation	Low	High	High
c-Myc	Low	High	High
Pax-2	Low	High	High
Clusterin	Low	High	High
E-Cadherin	High	Low	Low
HNF-1	High	Low	Low

Another prominent feature of the cyst epithelium is an abnormality in cell polarity (32). Recall that the plasma membrane of epithelial cells in the kidney and other organs

can be divided into a basolateral membrane (facing the blood) and an apical membrane (facing the urinary space). The border between the apical and basolateral membranes is produced by the tight junctions. The apical and basolateral membranes contain distinct sets of proteins. For example, the Na,K-ATPase (sodium pump) is located only in the basolateral membrane and not in the apical membrane, whereas sodium channels are present only in the apical membrane. The maintenance of separate apical and basolateral plasma membrane domains is absolutely vital for proper functioning of epithelial cells. For example, vectorial transport of sodium in the collecting duct requires sodium channels in the apical membrane operating in series with the Na,K-ATPase in the basolateral membrane. If the proteins were instead randomly distributed in the plasma membrane, no net transepithelial sodium transport would occur.

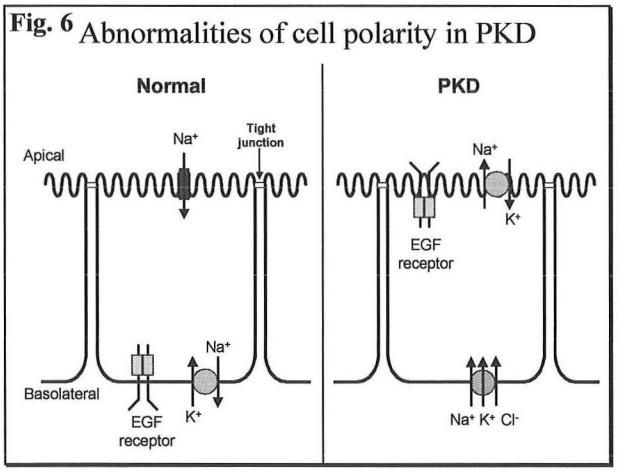


Fig. 6 Abnormalities of cell polarity in PKD

Studies first performed by Pat Wilson at Mt. Sinai School of Medicine have shown that ADPKD is characterized by an abnormality in cell polarity (Fig. 6) (33). Rather than being located in the basolateral plasma membrane, as it is in all renal tubules, the Na,K-ATPase is instead mislocalized in the apical plasma membrane (of at least some renal cysts). Another basolateral membrane protein, the receptor for epidermal growth factor (EGF), is also mislocalized in the apical membrane. There is not a generalized defect in cell polarity, since almost all apical membrane proteins are present where they should be. Rather, there seems to be a specific defect in which certain basolateral membrane proteins, but not others, are mislocalized in the apical membrane. The apical mislocalization of the Na,K-ATPase pump may be involved in transforming renal tubules from a normally absorptive epithelium to a secretory epithelium in which active secretion of salt and water contributes to cyst expansion. Recent studies provide a plausible mechanism for how abnormalities in cell polarity may be generated in ADPKD (see below).

Genetics

The familial nature of PKD was recognized around the turn of the century. Cairns described vertical transmission of PKD in 1925 and proposed that the disease was inherited as an autosomal dominant trait (34). Although PKD had been recognized as an inherited disease for almost a century, it was not until the late 20th century that tools became available to identify the disease gene. The discovery of the PKD1 gene in 1993 is a classic example of positional cloning. The steps in positional cloning include linkage analysis, physical mapping, and identification of the disease gene. Linkage analysis refers to the analysis of the inheritance of genetic markers in affected families. The goal of

linkage analysis is to identify genetic markers that segregate with the disease in families and are therefore closely linked to the disease. Ideally, one would like to identify flanking genetic markers, one located centromeric to the disease gene and one located telomeric to the disease gene, so that the disease gene must be located in the intervening DNA between the two markers. Next, physical mapping is performed in which large pieces of DNA that contain the genetic markers are assembled. The notion here is that if the genetic marker and disease are closely linked, then a large piece of DNA containing the known genetic marker may also contain the unidentified disease gene. Nowadays, this step has been greatly facilitated by the availability of the complete sequence of the human genome. The last step is to analyze the sequence of the DNA to identify potential genes and gene mutations.

The first breakthrough in the genetics of ADPKD occurred in 1985 when Steve Reeders, then at Oxford, showed that ADPKD was linked to the alpha-globin locus on human chromosome 16p (35). After Reeders moved to Yale, he and two of his renal fellows, Greg Germino and Steve Somlo, identified additional genetic markers that were more closely linked to the disease. Physical mapping of the ADPKD gene region began in 1988, and by 1992 a long-range physical map of the region had been assembled. Unfortunately, the region was 600 kb long and rich in genes. Thus, it was difficult to identify the disease gene by inspection. As frequently happens in positional cloning, the identification of the disease gene depended on serendipity. The next breakthrough occurred in 1993 and was reported in Cell in 1994 by Peter Harris and his colleagues at Oxford (36). They identified a unique family in which a woman with PKD had a son who developed PKD and a daughter who developed a distinct disorder, tuberous sclerosis. Cytogenetic analysis of the family revealed that the mother had a balanced translocation affecting chromosomes 16p and 22. Her son with PKD inherited the same balanced translocation, whereas her daughter with tuberous sclerosis had an unbalanced translocation in which the tip of chromosome 16p had been lost. Since the mother and son had balanced translocations, they carried a normal complement of genes, and it was hypothesized that their disease was due to the gene that was disrupted by the translocation. Subsequent cloning revealed that the translocation breakpoint interrupted a novel gene, which has now been named PKD1. The daughter developed tuberous sclerosis because the tip of chromosome 16p that was lost contained the TSC2 gene. (The relationship between the PKD1 and TSC2 genes is discussed in greater detail below.)

The PKD1 gene

The PKD1 gene is very large, consisting of 46 exons distributed over 50 kb. The gene encodes a 14.1 kb mRNA that is translated into a protein composed of 4302 amino acids. The initial analysis of the structure of the PKD1 gene revealed two striking features of the gene. The first was that the majority of the PKD1 gene is duplicated elsewhere on chromosome 16p. The duplicated region involves most of the 5' end of the gene extending to exon 26, which is located in the membrane domain. This region is duplicated at least 2-3 times at more centromeric sites on chromosome 16p. Moreover, the duplicated genes (termed HG) appear to be expressed as mRNA transcripts and may also encode proteins. The presence of the gene duplication has hindered mutational

analysis and may also be involved in the mechanism of mutagenesis via the process of gene conversion. The gene duplication may be unique to humans, since only a single copy of the PKD1 gene has been identified in mice. The second unique feature of the PKD1 gene was the presence of a 2.5 kb microsatellite repeat in intron 21. This microsatellite, which consists of repeats of the sequence (CCT)n is the longest known microsatellite repeat in the human genome. Since repeat sequences can predispose to mutations, the presence of the intron 21 sequence may also contribute to mutagenesis of the PKD1 gene.

As a consequence of the gene duplication, it has been very difficult to perform mutational analysis of the PKD1 gene in affected individuals. Since the PKD1 and HG genes are highly homologous, it was difficult to be certain that one was detecting mutations of the authentic PKD1 gene rather than a mutation of one of the HG genes. The conventional approach to identify gene mutations would involve PCR amplification of the exons followed by SSCP analysis or direct DNA sequencing. Since the duplicated region includes the exons and introns, it was initially not possible to design PCR primers that would specifically amplify PKD1 exons rather than HG exons. Thus, most mutations of the PKD1 gene that have been described are in the 3' unique region of the gene. More recently, with the use of long-range PCR and the protein truncation test (PTT), both Germino's group at Hopkins and Peter Harris' group, now at Mayo Clinic, have been able to identify mutations in the duplicated region of the PKD1 gene (37, 38). The first noteworthy observation is that many different mutations have been described in different families. Thus, unlike cystic fibrosis in which a single mutation of CFTR occurs in 70% of affected individuals, there are many different PKD1 mutations that can cause disease. Second, the mutations are located throughout the gene. Thus, there is not a hot spot for mutations. Third, different types of mutations have been observed including splice site, in-frame and out of frame deletions and insertions, nonsense mutations, and Missense mutations. The out of frame deletion/insertions and nonsense mutations are very likely to be inactivating mutations of the gene. Distinguishing missense mutations from nonpathogenic polymorphisms is more difficult. Since the biological function of polycystin-1 is not known, there is not a bioassay that could be performed to test the effects of mutations on polycystin-1 function. Nevertheless, missense mutations that affect highly conserved amino acids, e.g., cysteine residues in the PKD repeats, are also very likely to be relevant disease mutations. Mutations have now been found in the amino-terminal extracellular domain of polycystin-1, highlighting the possible importance of this region possibly as a receptor (see below).

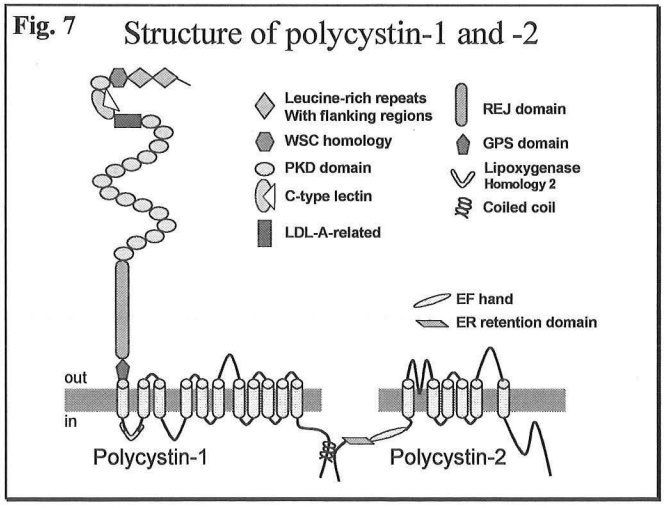
The PKD2 gene

The second PKD gene, PKD2, was cloned by Steve Somlo, then at Albert Einstein (39). While he was a nephrology fellow in Steve Reeder's laboratory, Somlo mapped the PKD2 locus to human chromosome 4. He then pursued conventional physical mapping and narrowed down the disease interval. After the PKD1 gene was identified, he searched the critical interval for a homologous gene, reasoning that the genes might be similar since the disease phenotypes were nearly identical. He identified a gene that was roughly 25% homologous to a region of PKD1. The gene, named PKD2, encodes a 5.3

kb mRNA that is translated into a protein consisting of 968 amino acids. Mutational analysis revealed that affected patients were heterozygous for inactivating mutations of the gene. Like PKD1, mutations of PKD2 are located throughout the gene. The PKD2 gene is not duplicated, which has simplified the mutational analysis.

Polycystins

The proteins encoded by the PKD1 and PKD2 genes define a new family of proteins, named the polycystins, which play important roles in biological processes ranging from fertilization to ion translocation to vesicle transport (40-43). Polycystin-1, the product of the PKD1 gene, is a huge protein containing 4302 amino acids with a molecular weight of about 500,000 daltons (44, 45). Fig. 7 shows a schematic diagram of the predicted structure of polycystin-1. The protein is an integral plasma membrane protein containing 11 transmembrane segments. The carboxyl-terminus is cytoplasmic and has been shown to interact with a number of cellular proteins, including cadherins, etc. The most distinctive aspect of the protein is the large, extracellular amino-terminal domain, which contains many distinct protein motifs including leucine-rich repeats, C-type lectin domain, a WSC domain, and 16 immunoglobin-like domains, called PKD repeats. Many of these motifs are involved in protein-protein or protein-carbohydrate interactions, raising the possibility that polycystin-1 may function as a receptor for an as yet unidentified ligand. Nearer the membrane, there is a region of homology to a sea urchin egg receptor protein and a potential proteolytic cleavage site (GPS domain). Between the first and second transmembrane domains there is a region of similarity to lipoxygenase 2 (PLAT domain). The C-terminus contains a coiled-coil domain that may also be involved in protein-protein interactions as well as numerous potential phosphorylation sites for various protein kinases.



Polycystin-1 is widely expressed in many tissues, including the kidney, brain, heart, bone, muscle, etc. (46). Within the kidney, polycystin-1 is found on the basolateral plasma membrane of tubular epithelial cells, especially collecting ducts. In immature tubules, expression is primarily on the basal membrane in focal adhesions that represent sites of cell-matrix interaction, whereas in mature tubules expression is primarily in the lateral membrane at sites of cell-cell contact. The function of polycystin-1 was not immediately apparent from its structure, but its sites of expression suggested that it might be a receptor involved in cell-cell or cell-matrix interactions. In addition to the kidney, polycystin-1 is expressed in many tissues, consistent with the sites of involvement in

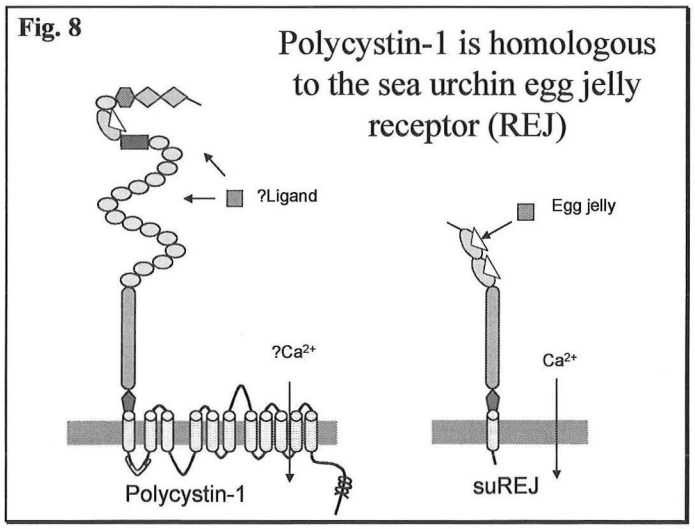
ADPKD. Polycystin-1 is expressed in smooth muscle cells in blood vessels, biliary tract epithelium, pancreas, and colonic smooth muscle cells.

Polycystin-2 is a much smaller protein of 968 amino acids that is also predicted to be an integral membrane protein (39). Polycystin-2 contains six transmembrane segments and amino- and carboxyl-termini that are both predicted to be intracellular (Fig. 7). The six transmembrane segments of polycystin-2 are similar to six of the 11 transmembrane segments of polycystin-1. Importantly, polycystin-2 is similar in structure to known channel proteins that are involved in transport of calcium and sodium. This observation suggested that polycystin-2 might function as an ion channel. Polycystin-2 is also widely expressed in many tissues, particularly the kidney, heart, ovary, testis, vascular smooth muscle, and small intestine (47). In the kidney, polycystin-2 is coexpressed with polycystin-1 in tubular epithelial cells. However, the subcellular localization of polycystin-2 may be somewhat different from polycystin-1 (48). Whereas polycystin-1 is primarily expressed in the basolateral cell membrane, polycystin-2 appears to be most highly expressed in the endoplasmic reticulum (49). Like polycystin-1, polycystin-2 is expressed in a number of extrarenal tissues that are involved in ADPKD including the heart, vascular smooth muscle, etc.

Since the original cloning of polycystin-1 and polycystin-2, homologous proteins that belong to the polycystin family have been identified in species ranging from sea urchin to roundworm to mice and humans. Although the biological functions of polycystin-1 and polycystin-2 are still not fully understood, three major hints to their functions have been provided by studies of these homologous proteins. These studies also illustrate how basic research in seemingly unrelated areas can have a major impact on the understanding of human disease.

Hint #1. Fertilization

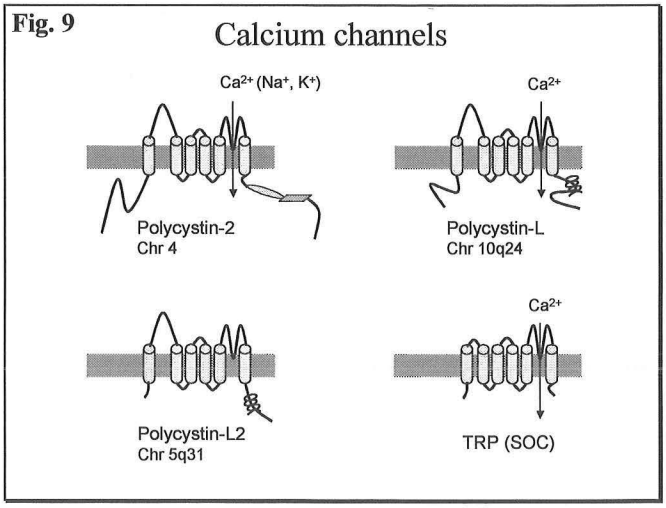
Polycystin-1 is similar in structure to the egg jelly receptor, a protein that is important in the fertilization of the egg by sperm. Prior to fertilization, sperm must penetrate a layer of extracellular matrix that surrounds the plasma membrane of the egg. To facilitate this penetration, sperm that contact the matrix undergo a process known as the acrosome reaction. The acrosome reaction represents a form of exocytosis in which a large vesicle contained in the sperm head fuses with the plasma membrane of the sperm and releases its contents into the surrounding extracellular medium. The vesicle contains enzymes that hydrolyze the matrix surrounding the egg and



permit the sperm to penetrate the matrix. Once the sperm reaches the egg plasma membrane it fuses and releases its DNA. The acrosome reaction is a critical step in fertilization and has been extensively investigated. These studies have revealed that the reaction is triggered by the interaction of a glycoprotein ligand in the egg jelly with a receptor in the plasma membrane of the sperm head. Ligand binding results in activation of an ion channel that permits the influx of calcium from the extracellular medium into the interior of the sperm. The spike in intracellular calcium concentration triggers exocytosis. It is not yet known whether the calcium channel activity and the receptor activity are mediated by the same protein or by different proteins. Recently, the egg jelly receptor from sea urchin sperm was cloned and found to be homologous to polycystin-1 (Fig. 8) (50). The extracellular domain of polycystin-1 contains C-type lectin domains, which bind carbohydrates, as well as a domain near the membrane that is homologous to the sea urchin egg jelly receptor. These studies suggest that polycystin-1 may also function as a receptor for an extracellular ligand, and that receptor activation might result in activation of calcium influx.

Hint #2. Calcium channels

The structure of polycystin-2 suggested that it might function as a cation channel. Two homologues of polycystin-2 have been identified in mammals, polycystin-L and polycystin-L2 (51-53) (Fig. 9). Neither protein is linked to ADPKD. However, when polycystin-L was expressed in Xenopus oocytes, it was found to conduct cations, including calcium (54). Polycystin-2 and polycystin-L share structural features with other voltage-activated calcium and sodium channels, as well as the transient receptor potential (TRP) channels. TRP channels are store-operated calcium channels that are activated in response to intracellular store depletion. Despite these similarities, it was initially not possible to identify channel activity on the plasma membrane of cells expressing polycystin-2. A major clue was provided by the realization that most of the polycystin-2 protein is present in the endoplasmic reticulum and therefore was not accessible to study from the plasma membrane. More recently, using a technique known as planar lipid bilayers, it has been possible to shown that polycystin-2 also functions as a calcium channel (55). Another major finding was that polycystin-2 directly interacts with polycystin-1. As discussed previously, both proteins are frequently found in the same cells, including kidney tubule cells. Studies have shown that the carboxyl-terminal domains of polycystin-1 and polycystin-2 interact through a structure known as a coiled-coil (in polycystin-1) (56, 57). Moreover, co-expression of polycystin-1 with polycystin-2 results in the appearance of calcium channels on the



plasma membrane of the cell (58). Taken together, these results suggest that polycystin-1 and polycystin-2 can assemble together to form a functional ion channel on the plasma membrane. The observation that the proteins interact functionally in the same pathway explains why mutations of either gene produce diseases with identical phenotypes.

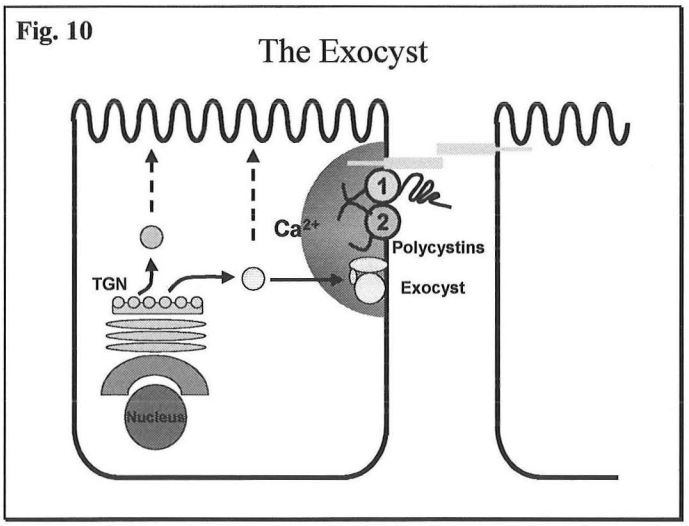
Hint #3. Mucolipidosis, type IV

A third hint to the function of polycystins was provided from an unexpected source, a lysosomal storage disease known as mucolipidosis, type IV. Mucolipidosis IV is an autosomal recessive disorder that is characterized by corneal clouding and psychomotor retardation. It is particularly prevalent among Ashkenazi Jews. Most lysosomal storage diseases are due to the deficiency of the enzymes that are required for digestion of lysosomal contents. However, in mucolipidosis IV the defect is in vesicle trafficking affecting late stages of endocytosis (59). As a result, lipids and proteins accumulate in lysosomes. Recently, the mucolipidosis IV gene was cloned and was found to encode a protein homologous to polycystin-2 named mucolipin-1 [Bargal, 2000 #3099; Bassi, 2000 #3100; (60)]. The counterpart of mucolipidin-1 in the roundworm (Cup-5) has been shown to be required for vesicle transport (61). Taken together, these studies suggest that polycystins are calcium channels that are involved in vesicle transport.

The exocyst

How do abnormalities in vesicle transport produce the abnormalities that are observed in cystic epithelium, such as the abnormalities in cell polarity? It turns out that the trafficking of proteins to the basolateral membrane depends on vesicle transport. In the apical border of the lateral membrane, near the cadherin-based adherens junctions, there is a structure known as the exocyst. First discovered in yeast by Peter Novick's group at Yale, the exocyst is a multiprotein complex that is involved in exocytosis. Studies from James Nelson's laboratory at Stanford have shown that in polarized epithelia, such as renal tubular epithelium, the exocyst is critical for formation of the basolateral membrane (62). The exocyst is thought to represent a "targeting patch" for fusion of vesicles containing proteins and lipids that are destined for the basolateral membrane. It does not appear to be involved in apical membrane trafficking. In PKD, the exocyst is severely inhibited (63). Taken together, these studies suggest a model for polycystin-1/2 function that is shown in Fig. 10 (64). In mature tubular epithelial cells, polycystin-1 is localized to the apical border of the lateral membrane, in a complex with E-cadherin and adjacent

Fig. 10



to the exocyst (65). Polycystin-2 is colocalized with polycystin-1 in the plasma membrane or resides in the subjacent endoplasmic reticulum. Activation of polycystin-1, perhaps via cell-cell contact or binding of an unidentified extracellular ligand, results in activation of polycystin-2 through protein-protein interaction. Together (and perhaps individually as well), polycystin-1/2 form an ion channel that when activated permits the entry of calcium (from the cell exterior or the interior of the endoplasmic reticulum). The **local** rise in calcium concentration permits vesicles containing basolateral membrane proteins to fuse with the exocyst and enter the basolateral membrane. In ADPKD, mutations of polycystin-1 or polycystin-2 block exocytosis to the basolateral membrane. Proteins such as the Na,K-ATPase pump and the EGF receptor that are normally targeted to the basolateral membrane end up instead in the apical membrane (there is always some transport to the apical membrane, but normally it is overwhelmed by the basolateral transport). The aberrant protein targeting lead to abnormalities in transepithelial transport (secretion instead of absorption) and abnormal cell growth (via activation of the EGF receptor).

ADPKD is a focal disease

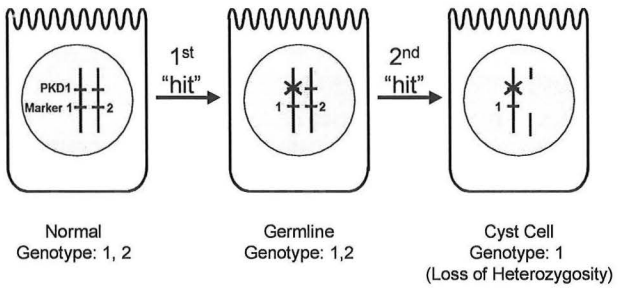
A seminal study was performed by Luc Baert who obtained kidneys from young adults with ADPKD at an early stage of disease (66). He microdissected complete nephrons from the kidneys to identify the source and extent of the cysts. Cysts arose from the tubular portion of the nephron as well as the renal collecting system. Moreover, although all cells of the nephron presumably carried the same germline mutation, only a few cysts arose per nephron. Many nephrons were dissected that appeared completely normal. Therefore, ADPKD is a **focal** disease that affects only a small minority of cells in the kidney, even though all cells carry the mutated gene.

I would now like to discuss the mechanism by which mutations of PKD1 and PKD2 cause disease. ADPKD is an autosomal dominant trait. Thus, affected individuals carry both a mutant and wild-type allele. Why is the wild-type allele unable to compensate for the mutant allele? As for any autosomal dominant disease, three mechanisms have been proposed. The first is haploinsufficiency. Most genes (except those that are imprinted or on the X chromosome) are expressed from both alleles. Thus, to a first approximation, inactivating mutations of one allele might result in a 50% reduction of the gene product, which could result in disease, particularly if the gene encodes a structural protein. The second mechanism is dominant-negative mutants. The mutant allele may encode a protein that interferes with the function of the normal protein encoded by the wild-type allele. The third possibility is that the mutations are actually recessive at the cellular level. In this mechanism, a mutant allele of, say PKD1, is inherited from an affected parent and a wild-type allele is inherited from the unaffected parent. During somatic life, i.e., after the zygote stage, the wild-type allele undergoes a somatic mutation. Consequently, the cells in which this second hit have occurred (and all progeny cells) will be homozygous for PKD1 mutations. Complete loss of PKD1 then leads to cyst formation. This situation is very analogous to Knudson's two-hit hypothesis of tumorigenesis. Fig. 11 (middle panel) shows the cells of an affected individual who has inherited one mutant allele from one parent and a normal allele from the unaffected

parent. Near the disease gene is a genetic marker. The individual is heterozygous at the marker, carrying allele 1 on the disease chromosome and allele 2 on the wild-type chromosome. The right panel shows that in some somatic cells there occurs a mutation of the wild-type chromosome, typically a large deletion. Since the deletion involves the nearby genetic marker, the wild-type allele 2 has been lost. Consequently, the cells appear to be homozygous for allele 1 (loss of heterozygosity, LOH).

Fig. 11

Knudson's two-hit hypothesis



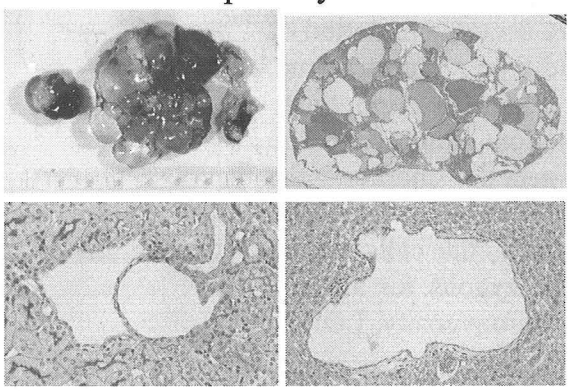
Recent studies have provided evidence favoring a two-hit model of cystogenesis in ADPKD (67). However, it is important to stress that the two other possibilities have not been excluded, and in fact, multiple mechanisms may be involved. Evidence for a cellular recessive model includes the following: 1) Renal cysts are clonal, consistent with their origin from a single cell undergoing a somatic mutation (68). 2) Two studies have shown that renal cysts exhibit loss of heterozygosity. Reduction to homozygosity is due to loss of the wild-type allele (68, 69). 3) Somatic mutations have been detected in cyst epithelium in both kidney and liver (68, 70-72). 4) A prediction of the model is that homozygous mutations of PKD1 and PKD2 would be more deleterious than heterozygous mutations. Indeed, no humans with homozygous mutations of either PKD1 or PKD2 have been observed, presumably because homozygosity is embryonic lethal.

Lessons from animal models

The PKD1 and PKD2 genes exist in the mouse genome. Knockout mice that lack one or both copies of the Pkd1 and Pkd2 genes have been created (73-77). Heterozygous mice do not develop cysts in the kidney or liver until late in life, whereas homozygous null mutant mice are embryonic lethal and develop severely cystic kidneys in utero. Kidney development in mice begins at 11.5 d.p.c. and proceeds normally until 14.5 d.p.c. Glomerular cysts first appear at 15.5 d.p.c., and by birth, the kidneys are massively replaced with cysts. Marker studies reveal that the cysts arise from all segments of the nephron and the renal collecting system. These results demonstrate that loss of Pkd2 or Pkd1 causes renal cysts.

Further evidence for the two-hit model comes from a unique strain of mice carrying a recombinigenic Pkd2 allele (called WS25) (73). The WS25 allele normally produces wild-type polycystin-2 protein. However, during somatic life and at a certain low frequency, a rearrangement occurs at the locus producing a null allele. Fig. 12 shows that mice that carry the WS25 allele develop cysts in the kidney and liver during adult life. This phenotype is identical to humans with ADPKD. Immunostaining with an antibody to polycystin-2 demonstrated staining in tubules but no expression of polycystin-2 in the cyst epithelium. This result indicates polycystin-2 expression is lost in cyst epithelium and strongly supports the two-hit hypothesis. WS25 mice represent the most authentic animal model of human ADPKD established to date.

Fig. 12 Adult Pkd2$^{WS25/-}$ mice develop renal and hepatic cysts



Studies of human ADPKD have also revealed loss of polycystin expression in cyst epithelium. However, only a fraction of cysts have absent expression (78). In many cysts, expression of polycystin is detected. Although it is possible that the second hits represent missense mutations that may not affect protein production, there may be other explanations for continued polycystin expression. Recently, it has been shown that cysts can exhibit trans-heterozygosity. That is, individuals that carry a germline mutation of PKD1 can acquire a second hit that involves the other PKD gene, PKD2 (79). Conversely, individuals carrying germline mutations of PKD2 can have cysts in which there are somatic mutations of PKD1 (80). These results suggest that haploinsufficiency of both PKD1 and PKD2 produces cysts. Trans-heterozygosity would explain why polycystin protein is still detectable in some cysts, since one allele of each gene is still expressed.

Tuberous sclerosis

Another situation in which polycystin expression is maintained in renal cysts is tuberous sclerosis (78, 81). Tuberous sclerosis is an autosomal dominant disorder characterized by benign hamartomas in multiple tissues, including the kidney, heart, brain, retina, liver, lung, bones, and skin. Clinical findings include hypopigmented macules, facial angiofibromas, cardiac rhabdomyomas, and cortical tubers and subependymal glial nodules in the brain (82). Seizures and mental retardation are common. The characteristic renal lesion is the angiomyolipoma, which is a benign tumor containing disorganized vascular, muscle, and adipose elements. Two genes that cause

tuberous sclerosis have been cloned: TSC1 on chromosome 9 and TSC2 on chromosome 16. The gene products, named hamartin and tuberin, respectively, appear to be tumor suppressors involved in the control of cell growth (83, 84). Patients with tuberous sclerosis also develop renal cysts (85). In particular, some patients with disease due to mutations of the TSC2 gene develop severe, early-onset PKD. The TSC2 gene is adjacent to the PKD1 gene in tail-to-tail orientation on chromosome 16. In 1994, it was shown that patients with TSC and early-onset PKD carried a chromosomal deletion that disrupted both the TSC2 gene and the adjacent PKD1 gene (Fig. 13) (86). This is a classic example of a contiguous gene deletion syndrome. Why should deletion of the adjacent TSC2 gene produce more severe disease than deletion of the PKD1 gene by itself? The answer may be found in a recent study suggesting that tuberin is required for the membrane localization of polycystin-1 (87). Cheryl Walker at Texas A&M has investigated the Eker rat, a rodent model of tuberous sclerosis carrying mutations of Tsc2. These studies have shown that Tsc2 heterozygous mutants develop PKD in adulthood due to second hits at the Tsc2 locus. Examination of cell lines that are deficient in the Tsc2 gene product, tuberin, revealed unexpectedly that polycystin-1 was mislocalized in the cells. In wild-type cells, polycystin-1 was localized appropriately in the basolateral membrane. However, in Tsc2 mutant cells, polycystin-1 was absent from the plasma membrane and instead accumulated in the golgi. These results demonstrate that tuberin is required for the transport of polycystin-1 to the plasma membrane. Therefore, humans or animals that carry germline mutations of both TSC2 and PKD1 are particularly susceptible to cyst formation.

Fig. 13

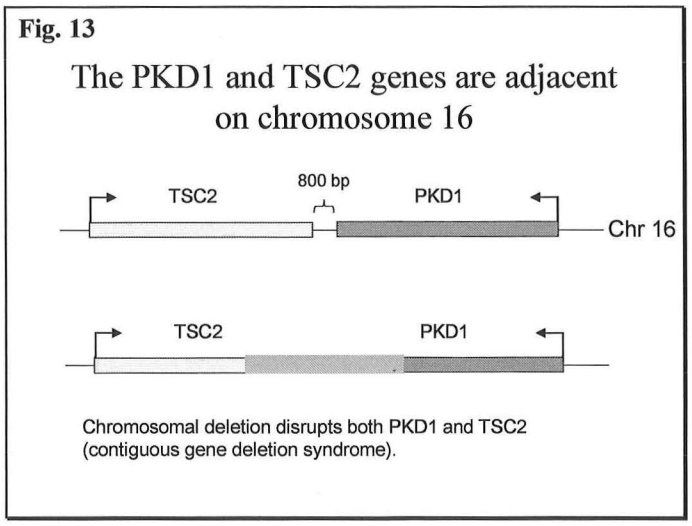
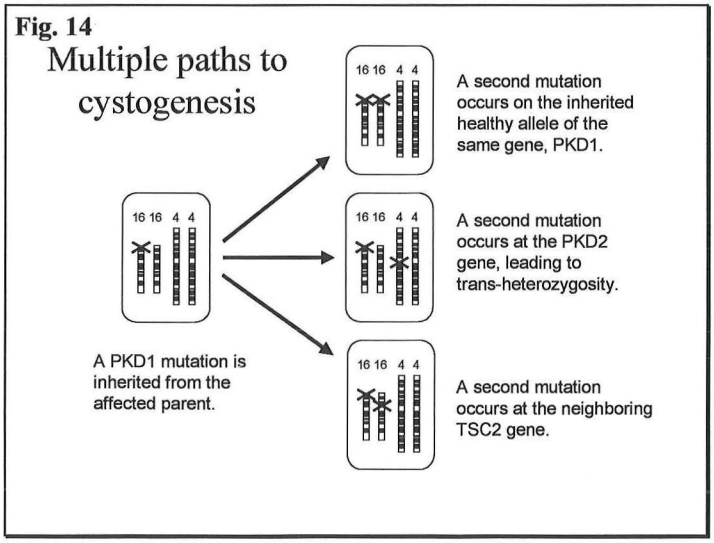


Fig. 14 summarizes three mechanisms by which mutations of PKD1 and PKD2 can cause disease. One mutation is inherited through the germline. During somatic life, a second hit occurs, which can be either a mutation of the same gene resulting in LOH or

Fig. 14



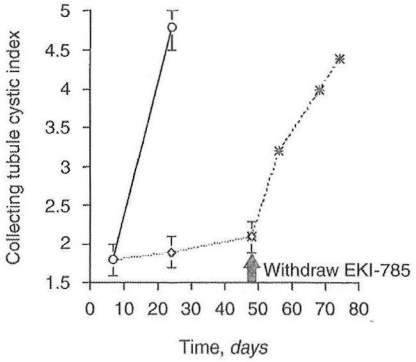
a mutation of the other PKD gene, resulting in trans-heterozygosity. Alternatively, germline or acquired mutations of both PKD1 and TSC2 can also cause cyst formation. The fact that compound mutations of PKD1 and PKD2 can cause disease suggests that the mechanism in these cells may be haploinsufficiency, in which decreased expression of two proteins in a common signaling pathway reaches a threshold that blocks signal transduction.

The EGF receptor is a novel target for therapy of PKD

Do the studies on the pathogenesis of PKD provide any opportunities for new treatments for the disease? One possible therapeutic target is the EGF receptor. As discussed above, one feature of polycystic kidney disease in both humans and mice is apical mislocalization of the EGF receptor. Activation of the mislocalized receptor by the high concentrations of TGF-α and EGF that are present in cyst fluid may contribute to cellular proliferation, which is a hallmark of the disease (see above). Recent studies in animal models provide compelling evidence for the role of EGF receptor activation in PKD. Transgenic mice that overexpress the EGF receptor ligand TGF-α develop renal cysts (88). In an elegant experiment performed by Ellis Avner and colleagues, mice with autosomal recessive PKD were mated with mice that carried a mutation of the EGF receptor (89). Mice that inherited both the PKD gene and the mutant EGF receptor exhibited less cyst formation than mutant mice that expressed the wild-type receptor. Based on the very promising results of targeted inhibition of the bcr/abl tyrosine kinase in the treatment of chronic myelogenous leukemia, there has been considerable interest in tyrosine kinase inhibitors that specifically inhibit the EGF receptor as possible therapies for PKD and carcinomas. EKI-785 is a small compound that irreversibly inactivates the EGF receptor by covalently binding to the ATP binding domain of the tyrosine kinase domain (90). Since receptor autophosphorylation is essential for activation, this compound blocks EGF receptor signaling. Fig. 15 shows that administration of EKI-785 to mice with PKD markedly inhibited cyst formation (91). Importantly, azotemia was prevented, and the lifespan of the animals was prolonged. These studies and others suggest that inhibition of the EGF receptor, either genetically or pharmacologically, ameliorates cyst formation both in vivo and in vitro (92). Pharmacologic blockade of EGF receptor signaling represents a promising therapy for human ADPKD. Phase I and phase II clinical trials of EGF receptor blockers are currently underway in humans.

Fig. 15 Inhibition of cyst formation with a tyrosine kinase inhibitor

	BUN	Cr
Control	19±2	0.2±0.1
bpk	191±31	0.6±0.2
bpk + EKI-785	19±4	0.2±0.1



Summary

In this review, we have seen the enormous progress since 1994 in understanding the pathogenesis of ADPKD, a common genetic disease of man since antiquity. The studies have identified a new protein family, the polycystins, which are involved in numerous biological processes including fertilization, ion translocation, and vesicle transport. Diseases that involve polycystins include lysosomal storage diseases and tuberous sclerosis as well as ADPKD. No effective therapy for ADPKD exists currently, but promising strategies are on the horizon, including EGF receptor blockade. The studies of ADPKD also illustrate how basic research in seemingly unrelated areas can converge to help us understand an important human disease.

Acknowledgments

Work from our laboratory is supported by the National Institutes of Diabetes, Digestive and Kidney Disease; the Yale Center for the Study of Polycystic Kidney Disease; and the PKD Foundation. I thank Steve Somlo (Yale), Jeff Cadeddu (Urology), Art Weinberg (Pathology), Joe Zhou (Pathology), and Steve Roach (Pediatrics) for providing material for this review. I also thank Dede Copeland for assistance with the preparation of the manuscript, and the members of my laboratory for helpful comments and suggestions.

References

1. Gabow, P. A. (1993) Autosomal dominant polycystic kidney disease. *New Engl. J. Med.* 329: 332-342
2. Fick, G. M., and Gabow, P. A. (1994) Hereditary and acquired cystic disease of the kidney. *Kidney Int.* 46: 951-964
3. Kimberling, W. J., Fain, P. R., Kenyon, J. B., Goldgar, D., Sujansky, E., and Gabow, P. A. (1988) Linkage heterogeneity of autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* 319: 913-918
4. Pei, Y., Paterson, A. D., Wang, K. R., He, N., Hefferton, D., Watnick, T. J., Germino, G. G., Parfrey, P., Somlo, S., and St. George-Hyslop, P. (2001) Bilineal disease and trans-heterozygotes in autosomal dominant polycystic kidney disease. *Am. J. Hum. Genet.* 68: 355-363
5. Chan, K. W. (1993) Adult polycystic kidney disease in Hong Kong Chinese: an autopsy study. *Pathology* 25: 229-232
6. Torres, V. E., and Watson, M. L. (1998) Polycystic kidney disease: antiquity to the 20th century. *Nephrol. Dial. Transplant* 13: 2690-2696
7. Fogazzi, G. B. (1998) The description of polycystic kidney by Domenico Gusmano Galeazzi. *Nephrol. Dial. Transplant* 13: 1039-1040
8. Hateboer, N., v Dijk, M. A., Bogdanova, N., Coto, E., Saggar-Malik, A. K., San Millan, J. L., Torra, R., Breuning, M., Ravine, D., and Group, f. t. E. P.-P. S. (1999) Comparison of phenotypes of polycystic kidney disease types 1 and 2. *Lancet* 353: 103-107

9. Ecder, T., and Schrier, R. W. (2001) Hypertension in autosomal-dominant polycystic kidney disease: early occurrence and unique aspects. *J. Am. Soc. Nephrol.* 12: 194-200
10. Torres, V. E., Cai, Y., Chen, X.-Z., Wu, G. Q., Geng, L., K.A., C., Johnson, C. M., and Somlo, S. (2001) Vascular expression of polycystin-2. *J. Am. Soc. Nephrol.* 12: 1-9
11. Torres, V. E. (1999) Extrarenal manifestations of autosomal dominant polycystic kidney disease. *Am. J. Kid. Dis.* 34: xlv-xlviii
12. Leier, C. L., Baker, P. B., Kilman, J. W., and Wooley, C. F. (1984) Cardiovascular abnormalities associated with adult polycystic kidney disease. *Ann. Int. Med.* 100: 683-688
13. Chapman, A. B., Johnson, A. M., Rainguet, S., Hossack, K., Gabow, P., and Schrier, R. W. (1997) Left ventricular hypertrophy in autsomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* 8: 1292-1297
14. Chauveau, D., Pirson, Y., Verellen-Dumoulin, C., Macnicol, A., Gonzalo, A., and Grunfeld, J. P. (1994) Intracranial aneurysms in autosomal dominant polycystic kidney disease. *Kidney Int.* 45: 1140-1146
15. Chapman, A. B., Rubinstein, D., Hughes, R., Stears, J. C., Earnest, M. P., Johnson, A. M., Gabow, P. A., and Kaehny, W. D. (1992) Intracranial aneurysms in autosomal dominant polycystic kidney disease. *New Engl. J. Med.* 327: 916-920
16. The Magnetic Resonance Angiography in Relatives of Patients with Subarachnoid Hemorrhage Study Group (1999) Risks and benefits of screening for intracranial aneurysms in first-degree relatives of patients with sporadic subarachnoid hemorrhage. *N. Engl. J. Med.* 341: 1344-1350
17. Huston, J., III, Torres, V. E., Wiebers, D. O., and Schievink, W. I. (1996) Follow-up of intracranial aneurysms in autosomal dominant polycystic kidney disease by magnetic resonance angiography. *J. Am. Soc. Nephrol.* 7: 2135-2141
18. Fick, G. M., Johnson, A. M., Strain, J. D., Kimberling, W. J., Kumar, S., Manco-Johnson, M. L., Duley, I. T., and Gabow, P. A. (1993) Characteristics of very early onset autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* 3: 1863-1870
19. Ravine, D., Gibson, R. N., Walker, R. G., Sheffield, L. J., Kincaid-Smith, P., and Danks, D. M. (1994) Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. *Lancet* 343: 824-827
20. Nicolau, C., Torra, R., Badenas, C., Vilana, R., Bianchi, L., Gilabert, R., Darnell, A., and Bru, C. (1999) Autosomal dominant polycystic kidney disease types 1 and 2: assessment of US sensitivity for diagnosis. *Radiology* 213: 273-276
21. Qian, Q., Harris, P. C., and Torres, V. E. (2001) Treatment prospects for autosomal-dominant polycystic kidney disease. *Kidney Int.* 59: 2005-2022
22. Modification of Diet in Renal Disease Study Group (1995) Dietary protein restriction, blood pressure control, and the progression of polycystic kidney disease. *J. Am. Soc. Nephrol.* 5: 2037-2047
23. Maschio, G., Alberti, D., Janin, G., Locatelli, F., Mann, J. F. E., Motolese, M., Ponticelli, C., Ritz, E., Zucchelli, P., and The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group (1996) Effect of the

angiotensin-converting-enzyme inhibitor Benazepril on the progression of chronic renal insufficiency. *N. Engl. J. Med.* 334: 939-945

24. Ecder, T., Edelsetin, C. L., Fick-Brosnahan, G. M., Johnson, A. M., Chapman, A. B., Gabow, P. A., and Schrier, R. W. (2001) Diuretics versus angiotensin-converting enzyme inhibitors in autosomal dominant polycystic kidney disease. *Am. J. Nephrol.* 21: 98-103
25. Elzinga, L. W., barry, J. M., Torres, V. E., Zincke, H., Wahner, H. W., Swan, S., and Bennett, W. M. (1992) Cyst decompression surgery for autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* 2: 1219-1226
26. McDougall, E. M. (1998) Role and long-term results of laparoscopic decortication in solitary cystic and autosomal dominant polycystic kidney disease. *J. Urol.* 159: 705-706
27. Brown, J. A., Torres, V. E., King, B. F., and Segura, J. W. (1996) Laparoscopic marsupialization of symptomatic polycystic kidney disease. *J. Urol.* 156: 22-27
28. Pearle, M. S., Traxer, O., and Cadeddu, J. A. (2000) Renal cystic disease. Laparascopic management. *Urol. Clin. North Am.* 27: 661-673
29. Nadasdy, T., Laszik, Z., Lajoie, G., Blick, K. E., Wheeler, D. E., and Silva, F. G. (1995) Proliferative activity of cyst epithelium in human renal cystic diseases. *J. Am. Soc. Nephrol.* 5: 1462-1468
30. Vanden Heuvel, G. B., Bodmer, R., McConnell, K. R., Nagami, G. T., and Igarashi, P. (1996) Expression of a *cut*-related homeobox gene in developing and polycystic mouse kidney. *Kidney Int.* 50: 453-461
31. Grantham, J. J. (1993) Fluid secretion, cellular proliferation, and the pathogenesis of renal epithelial cysts. *J. Am. Soc. Nephrol.* 3: 1843-1857
32. Wilson, P. D. (1997) Epithelial cell polarity and disease. *Am. J. Physiol.* 272: F434-F442
33. Du, J., and Wilson, P. D. (1995) Abnormal polarization of EGF receptors and autocrine stimulation of cyst epithelial growth in human ADPKD. *Am. J. Physiol.* 269: C487-C495
34. Cairns, H. W. B. (1925) Heredity in polycystic disease of the kidneys. *Q. J. M.* : 359-393
35. Reeders, S. T., Breuning, M. H., Davies, K. E., Nicholls, R. D., Jarman, A. P., Higgs, D. R., Pearson, P. L., and Weatherall, D. J. (1985) A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. *Nature* 317: 542-544
36. (1994) The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium [published errata appear in Cell 1994 Aug 26;78(4):following 724 and 1995 Jun 30;81(7):following 1170]. *Cell* 77: 881-894
37. Phakdeekitcharoen, B., Watnick, T. J., and Germino, G. G. (2001) Mutation analysis of the entire replicated portion of *PKD1* using genomic DNA samples. *J. Am. Soc. Nephrol.* 12: 955-963
38. Rossetti, S., Strmecki, L., Gamble, V., Burton, S., Sneddon, V., Peral, B., Roy, S., Bakkaloglu, A., Komel, R., Winearls, C. G., and Harris, P. C. (2001) Mutation analysis of the entire PKD1 gene: genetic and diagnostic implications. *Am. J. Hum. Genet.* 68: 46-63

39. Mochizuki, T., Wu, G., Hayashi, T., Xenophontos, S. L., Veldhuisen, B., Saris, J. J., Reynolds, D. M., Cai, Y., Gabow, P. A., Pierides, A., Kimberling, W. J., Breuning, M. H., Deltas, C. C., Peters, D. J. M., and Somlo, S. (1996) *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272: 1339-1342
40. Emmons, S. W., and Somlo, S. (1999) Mating, channels and kidney cysts. *Nature* 401: 339-340
41. Arnaout, M. A. (2001) Molecular genetics and pathogenesis of autosomal dominant polycystic kidney disease. *Annu. Rev. Med.* 52: 93-123
42. Harris, P. C. (1999) Autosomal dominant polycystic kidney disease: clues to pathogenesis. *Hum. Mol. Genet.* 8: 1861-1866
43. Wilson, P. D. (2001) Polycystin: new aspects of structure, function, and regulation. *J. Am. Soc. Nephrol.* 12: 834-845
44. (1995) Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. The International Polycystic Kidney Disease Consortium. *Cell* 81: 289-298
45. Sandford, R., Sgotto, B., Aparicio, S., Brenner, S., Vaudin, M., Wilson, R. K., Chissoe, S., Pepin, K., Bateman, A., Chothia, C., Hughes, J., and Harris, P. C. (1997) Comparative analysis of the polycystic kidney disease 1 (PKD1) gene reveals an integral membrane glycoprotein with multiple evolutionary conserved domains. *Hum. Mol. Genet.* 6: 1483-1489
46. Geng, L., Segal, Y., Pavlova, A., Barros, E. J., Lohning, C., Lu, W., Nigam, S. K., Frischauf, A. M., Reeders, S. T., and Zhou, J. (1997) Distribution and developmentally regulated expression of murine polycystin. *Am J Physiol* 272: F451-459
47. Obermuller, N., Gallagher, A. R., Cai, Y., Gassler, N., Gretz, N., Somlo, S., and Witzgall, R. (1999) The rat Pkd2 protein assumes distinct subcellular distributions in different organs. *Am. J. Physiol.* 277: F914-F925
48. Foggensteiner, L., Bevan, A. P., Thomas, R., Coleman, N., Boulter, C., Bradley, J., Ibraghimov-Beskrovnaya, O., Klinger, K., and Sandford, R. (2000) Cellular and subcellular distribution of polycystin-2, the protein product of the *PKD2* gene. *J. Am. Soc. Nephrol.* 11: 814-827
49. Cai, Y., Maeda, Y., Cedzich, A., Torres, V. E., Wu, G. Q., Hayashi, T., Mochizuki, T., Park, J. H., Witzgall, R., and Somlo, S. (1999) Identification and characterization of polycystin-2, the *PKD2* gene product. *J. Biol. Chem.* 274: 28557-28565
50. Moy, G. W., Mendoza, L. M., Schulz, J. R., Swanson, W. J., Glabe, C. G., and Vacquier, V. D. (1996) The sea urchin sperm receptor for egg jelly is a modular protein with extensive homology to the human polycystic kidney disease protein, PKD1. *J. Cell Biol.* 133: 809-817
51. Guo, L., Schreiber, T. H., Weremowicz, S., Morton, C. C., Lee, C., and Zhou, J. (2000) Identification and characterization of a novel polycystin family member, polycystin-L2, in mouse and human: sequence, expression, alternative splicing, and chromosomal localization. *Genomics* 64: 241-251
52. Nomura, H., Turco, A. E., Pei, Y., Kalaydjieva, L., Schiavello, T., Weremowicz, S., Ji, W., Morton, C. C., Meisler, M., Reeders, S. T., and Zhou, J. (1998)

Identification of PKDL, a novel polycystic kidney disease 2-like gene whose murine homologue is deleted in mice with kidney and retinal defects. *J Biol Chem* 273: 25967-25973

53. Wu, G., Hayashi, T., Park, J. H., Dixit, M., Reynolds, D. M., Li, L., Maeda, Y., Cai, Y., Coca-Prados, M., and Somlo, S. (1998) Identification of PKD2L, a human PKD2-related gene: tissue-specific expression and mapping to chromosome 10q25. *Genomics* 54: 564-568
54. Chen, X.-Z., Vassilev, P. M., Basora, N., Peng, J.-B., Nomura, H., Segal, Y., Brown, E. M., Reeders, S. T., Hediger, M. A., and Zhou, J. (1999) Polycystin-L is a calcium-regulated cation channel permeable to calcium ions. *Nature* 401: 383-386
55. Gonzalez-Perrett, S., Kim, K., Ibarra, C., Damiano, A. E., Zotta, E., Batelli, M., Harris, P. C., Reisin, I. L., Arnaout, M. A., and Cantiello, H. F. (2001) Polycystin-2, the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca^{2+}-mermeable nonselective cation channel. *Proc. Natl. Acad. Sci. USA* 98: 1182-1187
56. Qian, F., Germino, F. J., Cai, Y., Zhang, X., Somlo, S., and Germino, G. G. (1997) PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat Genet* 16: 179-183
57. Tsiokas, L., Kim, E., Arnould, T., Sukhatme, V. P., and Walz, G. (1997) Homo- and heterodimeric interactions between the gene products of PKD1 and PKD2. *Proc Natl Acad Sci U S A* 94: 6965-6970
58. Hanaoka, K., Qian, F., Boletta, A., Bhunia, A. K., Piontek, K., Tsiokas, L., Sukhatme, V. P., Guggino, W. B., and Germino, G. G. (2000) Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* 408: 990-993
59. Chen, C.-S., Bach, G., and Pagano, R. E. (1998) Abnormal transport along the lysosomal pathway in Mucolipidosis, type IV disease. *Proc. Natl. Acad. Sci. USA* 95: 6373-6378
60. Sun, M., Goldin, E., Stahl, S., Falardeau, J. L., Kennedy, J. C., Acierno, J. S., Jr., Bove, C., Kaneski, C. R., Nagle, J., Bromley, M. C., Colman, M., Schiffmann, R., and Slaugenhaupt, S. A. (2000) Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. *Hum. Mol. Genet.* 9: 2471-2478
61. Fares, H., and Greenwald, I. (2001) Regulation of endocytosis by CUP-5, the *Caenorhabditis elegans* mucolipin-1 homolog. *Nat. Gen.* 28: 64-67
62. Grindstaff, K. K., Yeaman, C., Anandasabapathy, N., Hsu, S.-C., Rodriguez-Boulan, E., Scheller, R. H., and Nelson, W. J. (1998) Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. *Cell* 93: 731-740
63. Charron, A. J., Nakamura, S., Bacallao, R., and Wandinger-Ness, A. (2000) Compromised cytoarchitecture and polarized trafficking in autosomal dominant polycystic kidney disease cells. *J. Cell Biol.* 149: 111-124
64. Somlo, S., and Ehrlich, B. (2001) Human disease: calcium signaling in polycystic kidney disease. *Curr. Biol.* 11: R356-R360

65. Huan, Y., and van Adelsberg, J. (1999) Polycystin-1, the *PKD1* gene product, is in a complex containing E-cadherin and the catenins. *J. Clin. Invest.* 104: 1459-1468
66. Baert, L. (1978) Hereditary polycystic kidney disease (adult form): A microdissection study of two cases at an early stage of the disease. *Kidney Int.* 13: 519-525
67. Qian, F., and Watnick, T. J. (1999) Somatic mutation as mechanism for cyst formation in autosomal dominant polycystic kidney disease. *Mol. Genet. Metab.* 68: 237-242
68. Qian, F., Watnick, T. J., Onuchic, L. F., and Germino, G. G. (1996) The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* 87: 979-987
69. Brasier, J. L., and Henske, E. P. (1997) Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. *J Clin Invest* 99: 194-199
70. Koptides, M., Hadjimichael, C., Koupepidou, P., Pierides, A., and Deltas, C. C. (1999) Germinal and somatic mutations in the *PKD2* gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum. Mol. Genet.* 8: 509-513
71. Watnick, T. J., Torres, V. E., Gandolph, M. A., Qian, F., Onuchic, L. F., Klinger, K. W., Landes, G., and Germino, G. G. (1998) Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. *Mol Cell* 2: 247-251
72. Pei, Y., Watnick, T. J., He, N., Wang, K., Liang, Y., Parfrey, P., Germino, G. G., and St. George-Hyslop, P. (1999) Somatic *PKD2* mutations in individual kidney and liver cysts support a "two-hit" model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* 10: 1524-1529
73. Wu, G., D'Agati, V., Cai, Y., Markowitz, G., Park, J. H., Reynolds, D. M., Maeda, Y., Le, T. C., Hou, H., Jr., Kucherlapati, R., Edelmann, W., and Somlo, S. (1998) Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* 93: 177-188
74. Wu, G., Markowitz, G. S., Li, L., D'Agati, V. D., Factor, S. M., Geng, L., Tibara, S., Tuchman, J., Cai, Y., Park, J. H., van Adelsberg, J., Hou, H., Jr., Kucherlapati, R., Edelmann, W., and Somlo, S. (2000) Cardiac defects and renal failure in mice with targeted mutations in *Pkd2*. *Nature Genet.* 24: 75-78
75. Kim, K., Drummond, I., Ibraghimov-Beskrovnaya, O., Klinger, K., and Arnaout, M. A. (2000) Polycystin 1 is required for the structural integrity of blood vessels. *Proc. Natl. Acad. Sci. USA* 97: 1731-1736
76. Lu, W., Peissel, B., Babakhanlou, H., Pavlova, A., Geng, L., Fan, X., Larson, C., Brent, G., and Zhou, J. (1997) Perinatal lethality with kidney and pancreas defects in mice with a targetted Pkd1 mutation. *Nat Genet* 17: 179-181
77. Lu, W., Fan, X., Basora, N., Babakhanlou, H., Law, T., Rifai, N., Harris, P. C., Perez-Atayde, A. R., Rennke, H. G., and Zhou, J. (1999) Late onset of renal and hepatic cysts in Pkd1-targeted heterozygotes [letter]. *Nat Genet* 21: 160-161
78. Ong, A. C. M., Harris, P. C., Davies, D. R., Pritchard, L., Rossetti, S., Biddolph, S., Vaux, D. J. T., Migone, N., and Ward, C. J. (1999) Polycystin-1 expression in

PKD1, early-onset PKD1, and TSC2/PKD1 cystic tissue. *Kidney Int.* 56: 1324-1333

79. Koptides, M., Mean, R., Demetriou, K., Pierides, A., and Deltas, C. C. (2000) Genetic evidence for a *trans*-heterozygous model for cystogenesis in autosomal dominant polycystic kidney disease. *Hum. Mol. Genet.* 9: 447-452
80. Watnick, T. J., He, N., Wang, K., Liang, Y., Parfrey, P., Hefferton, D., St. George-Hyslop, P., Germino, G. G., and Pei, Y. (2000) Mutations of *PKD1* in ADPKD2 cysts suggest a pathogenic effect of *trans*-heterozygous mutations. *Nat. Genet.* 25: 143-144
81. Sampson, J. R., Maheshwar, M. M., Aspinwall, R., Thompson, P., Cheadle, J. P., Ravine, D., Roy, S., Haan, E., Bernstein, J., and Harris, P. C. (1997) Renal cystic disease in tuberous sclerosis: Role of the Polycystic Kidney Disease 1 gene. *Am. J. Hum. Genet.* 61: 843-851
82. Au, K.-S., Rodriguez, J. A., Finch, J. L., Volcik, K. A., Roach, E. S., Delgado, M. R., Rodriguez, E., Jr., and Northrup, H. (1998) Germ-line mutational analysis of the TSC2 gene in 90 tuberous-sclerosis patients. *Am. J. Hum. Genet.* 62: 286-294
83. Potter, C. J., Huang, H., and Xu, T. (2001) *Drosophila Tsc1* functions with *Tsc2* to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell* 105: 357-368
84. Tapon, N., Ito, N., Dickson, B. J., Treisman, J. E., and Hariharan, I. K. (2001) The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. *Cell* 105: 345-355
85. Ewalt, D. H., Sheffield, E., Sparagana, S. P., DElgado, M. R., and Roach, E. S. (1998) Renal lesion growth in children with tuberous sclerosis complex. *J. Urol.* 160: 141-145
86. Brook-Carter, P. T., Peral, B., Ward, C. J., Thompson, P., Hughes, J., Maheshwar, M. M., Nellist, M., Gamble, V., Harris, P. C., and Sampson, J. R. (1994) Deletion of the *TSC2* and *PKD1* genes associated with severe infantile polycystic kidney disease--a contiguous gene syndrome. *Nat. Genet.* 8: 328-332
87. Kleymenova, E., Ibraghimov-Beskrovnaya, O., Kugoh, H., Everitt, J., Xu, H., Kiguchi, K., Landes, G., Harris, P. C., and Walker, C. (2001) Tuberin-dependent membrane localization of polycystin-1: a functional link between polycystic kidney disease and the *TSC2* tumor suppressor gene. *Mol. Cell* 7: 823-832
88. Lowden, D. A., Lindemann, G. W., Merlino, G., Barash, B. D., Calvet, J. P., and Gattone, V. H. I. (1994) Renal cysts in transgenic mice expressing transforming growth factor-α. *J. Lab. Clin. Med.* 124: 386-394
89. Richards, W. G., Sweeney, W. E., Yoder, B. K., Wilkinson, J. E., Woychik, R. P., and Avner, E. D. (1998) Epidermal growth factor receptor activity mediates renal cyst formation in polycystic kidney disease. *J. Clin. Invest.* 101: 935-939
90. Discafani, C. M., Carroll, M. L., Floyd, M. B., Jr., Hollander, I. J., Husain, Z., Johnson, B. D., Kitchen, D., May, M. K., Malo, M. S., Minnick, A. A., Jr., Nilakantan, R., Shen, R., Wang, Y.-F., Wissner, A., and Greenberger, L. M. (1999) Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with *in vivo* activity by N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785). *Biochem. Pharmacol.* 57: 917-925

91. Sweeney, W. E., Jr., Chen, Y., Nakanishi, K., Frost, P., and Avner, E. D. (2000) Treatment of polycystic kidney disease with a novel tyrosine kinase inhibitor. *Kidney Int.* 57: 33-40
92. Sweeney, W. E., Futey, L., Frost, P., and Avner, E. D. (1999) *In vitro* modulation of cyst formation by a novel tyrosine kinase inhibitor. *Kidney Int.* 56: 406-413