Polycystic Kidney Disease

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This is to acknowledge that Peter Igarashi, M.D. has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Igarashi will be discussing off-label uses in his presentation

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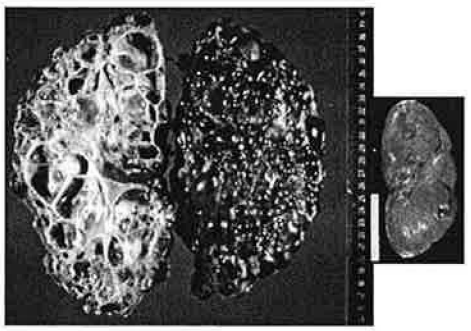
Introduction

Polycystic kidney disease (PKD) is the most common genetic cause of chronic kidney failure and one of the most common human genetic diseases overall. Approximately half of the affected individuals will develop end-stage renal disease (ESRD) requiring dialysis or transplantation. PKD is a systemic disorder with important extrarenal manifestations. Since this subject was last reviewed at Medical Grand Rounds in 2001, there have been major advances in our understanding of the molecular pathogenesis of PKD. Importantly, these new insights have, for the first time, led to the development of promising new therapies for PKD. In this grand rounds I will review the clinical manifestations of PKD and discuss how new concepts of pathogenesis have led to new therapeutic approaches. This review will focus on papers published since 2001. References to the older literature are provided in Ref. (21). I will focus on autosomal dominant PKD (ADPKD), which is the disease that primarily affects adults, and will not discuss autosomal recessive PKD (ARPKD), which primarily affects children.

PKD (ADPKD) affects 1/500 to 1/1,000 persons. This prevalence makes PKD more common than sickle-cell disease, cystic fibrosis, and Huntington's disease **combined**. PKD affects more than 600,000 individuals in the U.S. and more than 12.5 million people worldwide. Both sexes and all ethnic groups are affected equally. PKD primarily presents in middle-aged adults but occasionally presents in children and infants. PKD represents the fourth most common cause of ESRD in the U.S., accounting for 4–6% of patients on dialysis or requiring kidney transplantation. The direct medical costs of PKD have been estimated at more than $1.5 billion per year.

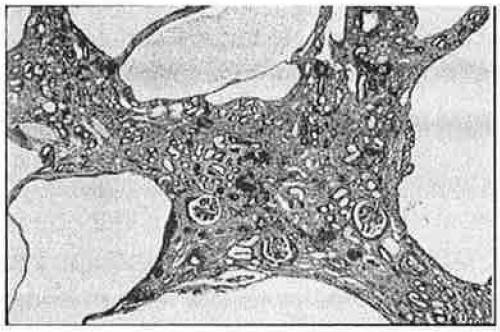
Pathology of PKD

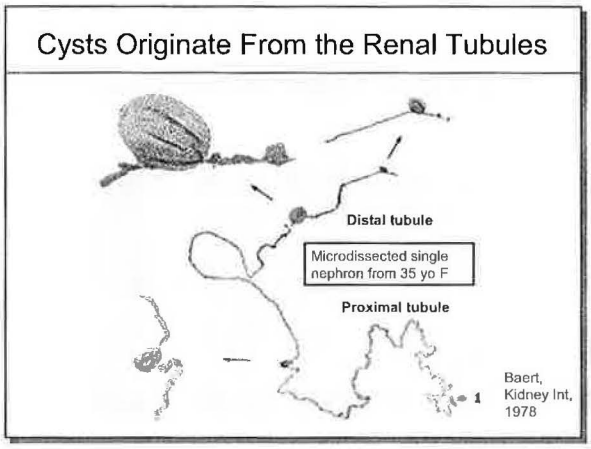


Courtesy of R. Perrone

PKD is characterized by the accumulation of numerous fluid-filled cysts in the renal parenchyma. The cysts grow progressively over time resulting in bilateral kidney enlargement that can be massive. The growth of the cysts compromises the adjacent normal nephrons leading to a progressive decline in kidney function. Histologically, the cysts are lined by a single layer of epithelial cells called the cyst epithelium. The kidney cysts are derived from renal tubules but eventually lose their connection to the tubule. The fluid within the cysts is produced by the cyst epithelial cells via a process of active secretion. In end-stage kidneys, the cysts are surrounded by areas of fibrosis containing atrophic tubules.

Histopathology of PKD





In 1978, Luc Baert reported a seminal study in which he microdissected individual nephrons from the kidneys of a 35 year-old woman with PKD who died of an unrelated cause (2). Even at this early age before any clinical evidence of kidney failure, cysts were found in the nephrons. The cysts could originate from any segment of the nephron. Moreover, cyst formation was focal, i.e., although all cells in the nephron carried the gene mutation, cysts arose only at discreet locations. It is now believed that cyst formation is focal because a second hit is required to initiate cyst formation. One gene mutation is inherited through the germline and a second mutation is acquired during life. Thus, at the cellular level PKD is a recessive disorder resulting from the loss of gene function.

Clinical Manifestations

The clinical manifestations of PKD can be divided into renal and extrarenal manifestations. The renal manifestations are due to the formation and growth of cysts throughout both kidneys and include pain, hematuria, cyst infection, impaired urinary concentrating ability, and progressive loss of kidney function.

Pain is the most common renal manifestation of PKD and affects 60% of adults and 35% of children. Patients can present with diffuse abdominal pain or flank pain. Acute pain arises from hemorrhage into a cyst, cyst infection, kidney stones, or cyst rupture. Chronic pain often manifests as back pain due to the severe lordosis produced by the massively enlarged kidneys. Treatment of chronic pain is difficult, and a stepwise approach has been recommended beginning with physical measures (ice massage, heating pads, whirlpool, Alexander technique), behavior modification, and analgesics (3). More severe cases may requires TENS, acupuncture, or spinal cord stimulation. Laparoscopic decompression of kidney cysts or nephrectomy may be required for refractory pain.

Gross or microscopic **hematuria** occurs in about 60% of patients with PKD. Hematuria arises from hemorrhage into a cyst that communicates with the renal collecting system. The walls of the kidney cysts are highly vascular due to the production of VEGF and other angiogenic factors by the cyst epithelial cells (55). Hematuria is usually managed conservatively with fluids, bedrest, and analgesics. Rarely transfusion, nephrectomy, and embolization are required.

Kidney **infection** occurs in 30-50% of individuals with PKD and manifests clinically as fever and flank pain. Patients may also present with point abdominal tenderness if they have a solitary infected cyst. It is important to note that the urine culture may be negative because cysts frequently do not communicate with the collecting system. Treatment consists of a prolonged course of a lipophilic antibiotic, such as ciprofloxacin, trimethoprim/sulfamethoxazole, or

chloramphenicol. Most penicillins and aminoglycosides do not penetrate adequately into the cyst fluid. Drainage is performed for refractory infections.

Kidney stones occur in approximately 20% of patients. Stones may contain primarily uric acid or calcium oxalate. Uric acid stone formation has been reported to be particularly common in PKD. Predisposing factors include hypocitraturia, hyperoxaluria, hyperuricosuria, hyperuricemia, and hypercalciuria. Patients with PKD may also have a distal acidification defect contributing to nephrolithiasis. In addition, the expanding cysts compress the renal collecting system producing urinary stasis which contributes to stone formation.

Kidney failure occurs in about 50% of patients with PKD. The overall rate of decline of GFR, as measured in the MDRD study, was 5–6 ml/min/yr in males and 4–5 ml/min/year in females. However, it is important to note that the course of PKD can be highly variable. Even individuals in the same family who have inherited the identical gene mutation can have very different rates of decline of GFR. This variability is thought to arise from both environmental and genetic factors. For example, recent studies from our laboratory have shown that acute kidney injury stimulates cyst formation in a mouse model of PKD (35). These results suggest that individual variation in exposure to nephrotoxins or subclinical kidney injury may underlie some of the variation that is seen in PKD. Poor prognostic indicators that have been identified include mutation of *PKD1*, male gender, early-onset hypertension, and increased proteinuria (6).

Kidney Volume Predicts Subsequent Decline in GFR

Kidney Volume	Change in GFR
<750 ml	1.39 ml/min/yr
750-1500 ml	–0.69 ml/min/yr
>1500 ml	–4.33 ml/min/yr

Grantham, NEJM, 2006

Another poor prognostic indicator that has recently been identified is increased **kidney size**. That increased kidney size predicts a more rapid decline in kidney function might be self-evident but was actually only established recently by a large NIH-funded study called CRISP (Consortium for Radiologic Imaging Studies of PKD) (14). CRISP enrolled 241 subjects with PKD and normal renal function at five centers in the U.S. Kidney and cyst volume were followed by annual MR imaging, and GFR was measured by iothalamate clearance. The patients were followed for three years. The CRISP study found that serum creatinine was a relatively poor measure of disease severity in PKD. Serum creatinine did not increase until the kidney volumes exceeded 1,500 ml (normal is 200 ml). Serial MR imaging showed that the kidney cysts grew exponentially over the 3-year period, and the baseline kidney volume predicted the subsequent decline in GFR. If the initial kidney volume was less than 750 ml the change in GFR over the 3-year period was +0.14 ml/min/year. In contrast, if the kidney volume was greater than 1500 ml, GFR declined at a rate of -4.3 ml/min/year. These results indicate that serial MR imaging can be useful for following the progression of PKD and that large kidney volume is a risk factor for more rapid disease progression. Because serum creatinine is a relatively poor measure compared to MRI, the FDA is currently being petitioned to accept decreased kidney volume as a measure of efficacy of investigational drugs.

Extrarenal Manifestations

The gene mutation that causes PKD is present in all cells in the body. Therefore, PKD should be considered a systemic disease that can have important extrarenal manifestations. In addition to kidney cysts, which are present in 100% of affected individuals, cysts are also found in other epithelial organs. Liver cysts are present in 75% of patients with PKD. The liver cysts originate from the bile ducts and can produce massive liver enlargement as well as symptoms such as pain. However, liver function tests are generally normal. This clinical picture contrasts with the autosomal recessive form of PKD in which liver fibrosis is complicated by portal hypertension. In addition to the kidney and liver, cysts are present at a lower frequency in the pancreas (10%), seminal vesicle, spleen, and choroid plexus.

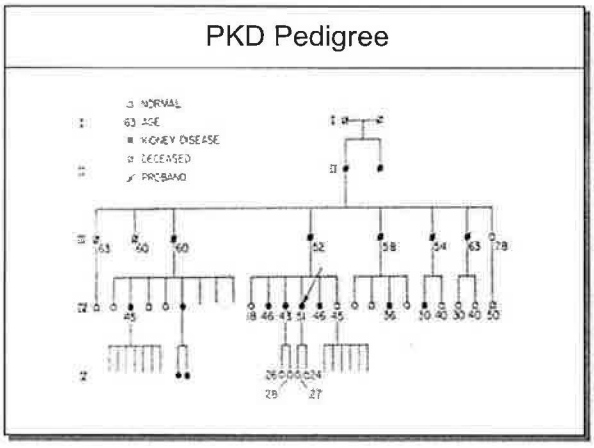
The most significant extrarenal manifestations of PKD involve the cardiovascular system. **Cardiac valvular abnormalities** include mitral valve prolapse and mitral and aortic regurgitation. Left ventricular hypertrophy is found even in individuals who do not have hypertension raising the possibility that LVH is a primary manifestation of PKD. This hypothesis is supported by animal studies showing that mutations of PKD genes produce cardiac abnormalities (15).

Intracranial aneurysms (ICA) are the most feared complication of PKD. The incidence of ICA is 4- to 12-fold higher in PKD patients than in the general population. The incidence is even higher (22%) if there is a positive family history (4). Most ICA affect the anterior circulation, and they are more common in individuals with mutations in the 5' end of the *PKD1* gene (40). Management of ICA has been a clinical conundrum. On the one hand, ICA have been documented to have a slow rate of progression (12). On the other hand, if an ICA ruptures it is associated with 50% mortality and 80% morbidity. There is no consensus on the management of aneurysms that are less than 10 mm in size. Larger aneurysms or those that produce symptoms are usually resected. ICA can be detected by MRA (magnetic resonance angiography), but routine screening is generally not recommended. Individuals who should be screened include those with a positive family history, individuals with prior subarachnoid hemorrhage, and those who have high-risk occupations such as airline pilots. In addition to aneurysms in the cerebral circulation, patients with PKD can also develop aneurysms in other large arteries including the aorta.

Hypertension is very common in PKD occurring in 66% of men and 41% of women. Hypertension is a primary manifestation of PKD since it occurs in 59% of individuals prior to any significant decline in GFR. Hypertension is associated with LVH and correlates with larger kidney and cyst volumes. The pathogenesis of hypertension is controversial. Older studies have emphasized the role of activation of the renin-angiotensin (RAS) system. The enlargement of the cysts was thought to compress the renal arteries resulting in ischemia, which stimulated renin release. Activation of the RAS provided the rationale for the treatment of hypertension with ACE inhibitors and ARBs preferentially. More recent studies, however, have shown that renin levels are not elevated in PKD suggesting either activation of the intrarenal RAS or a primary vasculopathy. As evidence for the latter, PKD gene products are expressed in vascular smooth muscle cells and endothelial cells and have been implicated in flow-dependent nitric oxide synthesis (33).

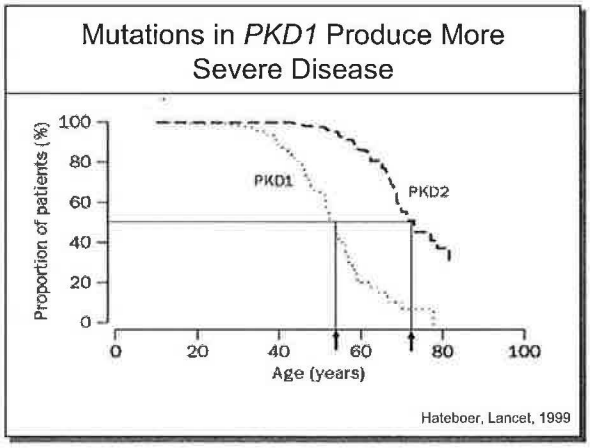
The optimal approach to the treatment of hypertension in PKD is controversial. In a nonrandomized study, an ACE inhibitor (enalapril) decreased LVH more than a calcium channel blocker (amlodipine) despite similar BP control (44). Enalapril also decreased proteinuria more than other antihypertensives, and proteinuria is a risk factor for progression of other chronic kidney diseases (8, 22). Diuretics may decrease renal function more than ACE inhibitors (9). However, no published studies have shown a clear benefit of ACE inhibitors or ARBs over other antihypertensive drugs. All studies have been too small to exclude a benefit, and all studies have been too short to exclude a long-term benefit. Accordingly, ACE inhibitors, ARBs, calcium channel blockers and diuretics may be used to treat hypertension in patients with PKD. The goal of antihypertensive therapy is also not clear. A secondary analysis of the MDRD study showed that patients with low BP (125/75) vs. usual BP (140/90) were 34% less likely to reach kidney failure (43). However, another study showed that aggressive blood pressure control reduced LVH but had no effect on renal function (44). To address these questions, the NIH is currently conducting a multicenter clinical study called **HALT-PKD** which will test whether the addition of an ARB to an ACE inhibitor reduces progression of PKD as measured by serial kidney volume (6). HALT-PKD will also compare usual vs. aggressive blood pressure control.

Genetics



PKD is a genetic disorder that is inherited as an autosomal dominant trait. There also exists a form of PKD that is inherited as an autosomal recessive trait. This form called ARPKD primarily affects infants and children and will not be discussed further here. The autosomal dominant form of PKD (ADPKD) primarily affects adults and can arise from mutations in either of two genes. The first gene is located on chromosome 16 and is called *PKD1*. Mutations of *PKD1* are responsible for about 85% of cases of PKD. *PKD1* encodes a protein called polycystin-1. About 15% of cases of ADPKD are caused by mutations of a different gene, called *PKD2*, which is located on chromosome 4. *PKD2* encodes a protein called polycystin-2.

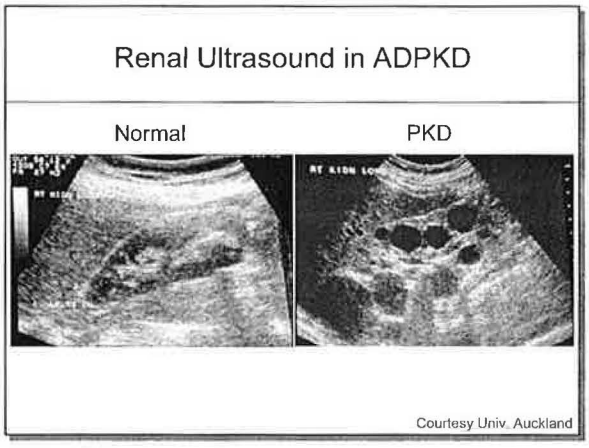
Mutations of *PKD1* and *PKD2* produce identical clinical manifestations except that mutations of *PKD2* produce less severe disease. Whereas patients with mutations of *PKD1* develop kidney failure at a median age of 53, patients with mutations of PKD2 do not develop kidney failure until a median age of 69, almost a 20 year difference (18). The explanation for the difference in severity has been provided by the CRISP study, which found that individuals with mutations of *PKD2* have smaller kidneys than



individuals with mutations of *PKD1*. The smaller size of the kidneys was due to a smaller number of cysts (17). Although the number of cysts was lower, the cysts grew at the same rate in both *PKD1* and *PKD2*.

Diagnosis

Given the massive enlargement of the kidneys that is seen in PKD, the diagnosis would seem to be straightforward and easily made by simple physical examination. Indeed, in end-stage PKD the enlarged kidneys are frequently palpable. However, diagnosis of PKD in younger individuals prior to the development of kidney failure can be challenging. It is also important to remember that there are many different genetic diseases that can produce kidney cysts. Although ARPKD primarily affects children, there are cases that do not present clinically until adolescence or adulthood, and these can be confused with ADPKD. Kidney cysts are also seen in tuberous sclerosis, von Hippel-Lindau disease, nephronophthisis, medullary cystic kidney disease, oro-facio-digital syndrome, glomerulocystic kidney disease, and renal cysts and diabetes. Many of these disorders have other clinical features that distinguish them from PKD. In addition, simple kidney cysts are common in the general population. Acquired kidney cysts are seen in patients with ESRD from any cause.



Ultrasound Criteria for ADPKD

Age	Positive Family History*	Negative Family History
< 30 yr	2 cysts	5 cysts bilateral
30-60 yr	4 cysts bilateral	5 cysts bilateral
>60 yr	8 cysts bilateral	8 cysts bilateral

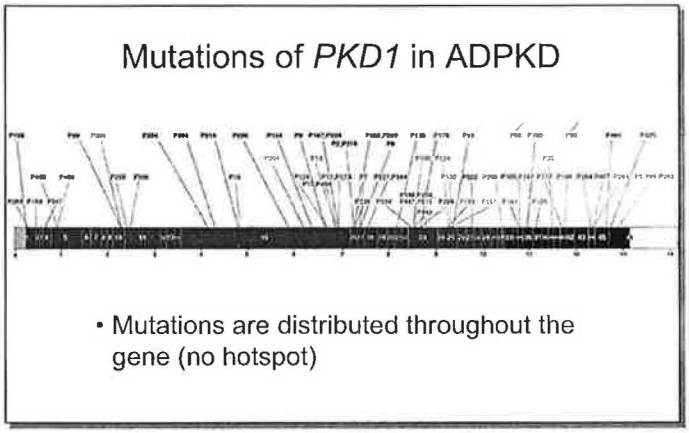
*Positive and negative predictive values 97-100%

Ravine, Lancet, 1994

The diagnosis of PKD is usually made by renal ultrasound, in which the cysts can be visualized as multiple, round, echolucent structures within the kidney. Because simple cysts are found in the general population and increase in number with age, the diagnostic criteria for PKD are age-dependent (39). For "at-risk" individuals who have a positive family history and are less than 30 years of age, two cysts in the kidney are sufficient to make a diagnosis. For individuals 30-60 years of age, four cysts are required, and they must be bilateral. Over age 60, 8 cysts and bilateral involvement are required. In individuals who do not have a positive family history, the number of cysts that are needed to make a diagnosis is higher: 5 cysts affecting both kidneys in individuals less than 60 and 8 cysts in individuals greater than 60. The positive and negative predictive value of these criteria is 97-100%. In addition to ultrasound, PKD can also be diagnosed with other imaging studies such as MRI and CT.

Genetic testing for PKD is available and is often requested by patients (6). Two types of genetic testing can be performed: linkage analysis and DNA sequencing. Linkage analysis requires blood from the individual as well as affected and unaffected family members, whereas

DNA-based testing can be performed with only blood from the individual. A complication of genetic testing is that the *PKD1* gene, which is most commonly mutated, is one of the largest genes in the human genome consisting of 46 exons distributed over more than 500,000 base pairs of DNA. In addition, the *PKD1* gene is duplicated several times elsewhere on chromosome 16. These duplicated genes are not involved in the pathogenesis of PKD but their presence makes DNA sequencing difficult because it is difficult to determine whether the sequence is from the authentic *PKD1* gene or one of the copies. This problem has been resolved with improvements in technology. In the U.S., the only company that is licensed to perform genetic testing for PKD is Athena Diagnostics (www.athenadiagnostics.com). The test is expensive, costing approximately $3,000, and may not be covered by insurance. Moreover, the test is only about 75% sensitive because of the large size of the *PKD1* gene. Indications for genetic testing include family planning, desire to know, and transplant donor evaluation. The latter refers to a situation where a patient with PKD develops ESRD and wishes to have a kidney transplant from a living-related donor. Genetic testing can determine whether the potential donor also carries the gene mutation.



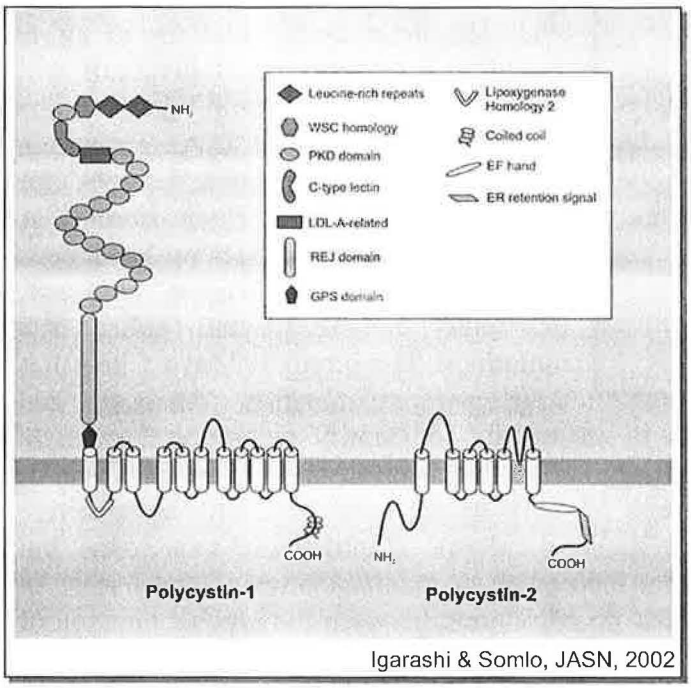
Genetic testing has revealed that PKD can arise from mutations of either *PKD1* or *PKD2*. Rarely, individuals will carry mutations of both genes. The mutations can be found anywhere within the genes, i.e., there is not a "hotspot" for mutation as there is in cystic fibrosis. A variety of different mutations have been identified including missense, nonsense, deletions, frameshift, and splice site mutations. The group at Mayo Clinic has developed a mutation database and performed **genotype-phenotype correlations** in an attempt to identify mutations that might predict clinical manifestations (41). In general, this effort has been disappointing. Analysis of *PKD1* has revealed that mutations in the 5' end of the gene are associated with more severe disease, earlier onset of kidney failure, and a higher incidence of intracranial aneurysms, but there are no specific mutations that can predict the course or manifestations of the disease in individual patients. Analysis of *PKD2* has found no correlation between the type or position of the mutation and disease severity.

Despite the availability of genetic testing, many individuals who have a positive family history have been reluctant to have genetic testing performed because of fear of negative repercussions for employment and health insurance. To address these concerns, the U.S. Congress just last week passed the **Genetic Information Nondiscrimination Act (GINA)**. Bills to prohibit genetic discrimination were first introduced 14 years ago. The GINA bill was introduced in the House on January 16, 2007 and passed the House on April 5, 2007 by a vote of 420-3. Despite this strong bipartisan support, the bill sat in the Senate for almost a year where it was blocked from a floor vote by a single senator. Earlier this year the objections of the senator were mitigated, and the bill passed the Senate on April 24, 2008 by a vote of 95-0. President Bush is expected to sign the bill into law next week. GINA protects Americans from discrimination based on genetic information. It prohibits health insurers from denying coverage

or adjusting premiums based on genetic information; prohibits employers from firing, refusing to hire, or discriminating based on genetic information; and prohibits employers from requiring individuals to undergo genetic testing. The enactment of GINA represents a major advance for the civil rights of individuals with PKD and other genetic diseases.

The Polycystins

The proteins that are encoded by the PKD disease genes, *PKD1* and *PKD2*, are named polycystin-1 and polycystin-2, respectively (21). **Polycystin-1** is a large membrane protein composed of 4,302 amino acids. Polycystin-1 contains a large extracellular domain, a membrane domain, and a short cytoplasmic tail. The extracellular domain contains an array of motifs involved in protein-protein interaction. A GPS domain near the membrane represents a site of proteolytic cleavage. The C-terminal cytoplasmic domain has been shown to interact with numerous proteins. Despite its identification more than 14 years ago, the function of polycystin-1 remains unknown. Its structure suggests that it may be a receptor for a ligand that has not yet been identified, perhaps polycystin-1 itself. Alternatively, polycystin-1 may function as a mechanosensor. Polycystin-1 has been shown to regulate numerous signaling pathways including Wnt/beta-catenin, G protein-coupled receptors, AP-1, mTOR, and JAK/STAT (57). Polycystin-1 has been localized in the plasma membrane, especially at sites of cell-cell contact, and in the primary cilium. Recent studies have shown that polycystin-1 undergoes regulated proteolysis similar to the protein, Notch. Cleavage at the GPS site is essential since a mutation that prevents cleavage produces kidney cysts (60). Proteolytic cleavage of the cytoplasmic side releases the C-terminal tail, which translocates to the nucleus where it may regulate gene transcription (7).



Knockout mice that lack polycystin-1 have been produced by numerous investigators (16). Homozygous null mutant mice that lack polycystin-1 are generally embryonic lethal due to abnormalities in cardiovascular development. *Pkd1* mutant embryos also develop cysts in the kidney and pancreas. Heterozygous mutant mice have a genotype that more closely resembles humans with PKD and develop cysts in the kidney and liver but with reduced severity compared to humans.

Polycystin-2, the product of the PKD2 gene, is composed of 968 amino acids. Like polycystin-1, polycystin-2 is an integral membrane protein. Polycystin-2 contains 6 transmembrane alpha-helices and belongs to the TRP family of ion channels (53). An alternative

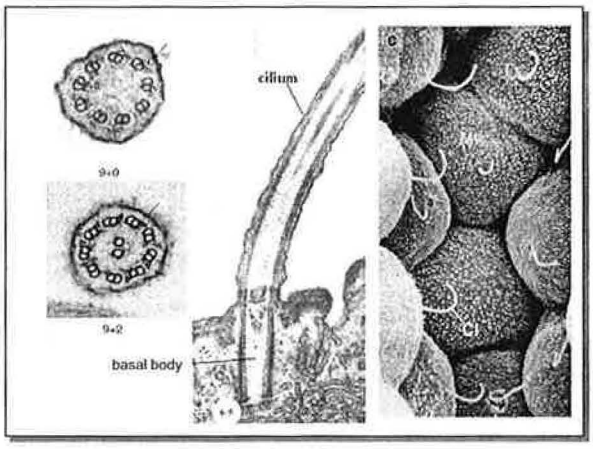
name for polycystin-2 is TRPP2. Studies have shown that polycystin-2 functions as a channel that conducts calcium ions. Polycystin-2 is primarily located in the endoplasmic reticulum but has also been detected in the primary cilium. Polycystin-2 and polycystin-1 physically interact suggesting that they function in a common pathway. This interaction may explain why mutations of either polycystin-1 or polycystin-2 produce identical phenotypes.

Knockout mice that lack polycystin-2 have also been generated and develop kidney cysts. In addition, *Pkd2* mutant mice show abnormalities in left-right asymmetry consistent with the role of primary cilia in left-right axis determination (36).

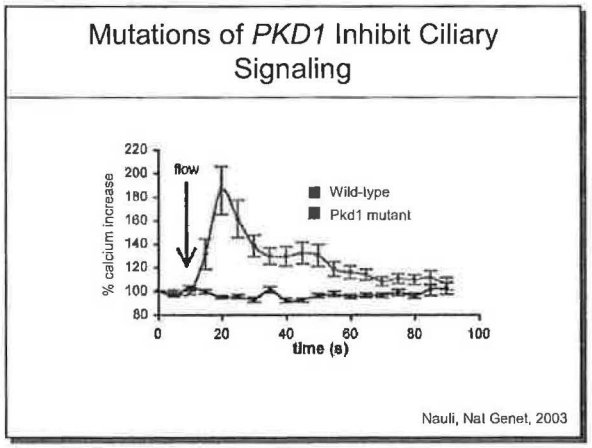
The Primary Cilium

Recent studies indicate that PKD is the founding member of a new class of diseases named the **ciliopathies**. These diseases are characterized by abnormalities in the structure or function of the primary cilium. In addition to PKD, the ciliopathies include nephronophthisis, retinitis pigmentosa, Bardet-Biedl syndrome, and oral-facial-digital syndrome (1, 34).

The primary cilium is a microtubule-based organelle that projects from the surface of most cells in the body (20, 21, 34). The primary cilium consists of an axoneme composed of microtubules surrounded by the ciliary membrane. The primary cilium is anchored in the cell body by the basal body. Cilia in the body can be classified into two major types based on the structure of their axonemes. Motile cilia, for example those in the respiratory tract, contain an axoneme composed of nine microtubule doublets surrounding two central microtubules (9+2 pattern). In contrast, primary cilia are non-motile and contain nine peripheral doublets lacking the two central microtubules (9+0 pattern). A single primary cilium can be found on the surface of most cells in the body except blood cells. In the kidney, a primary cilium projects from the apical surface of each epithelial cell into the lumen of the tubule.

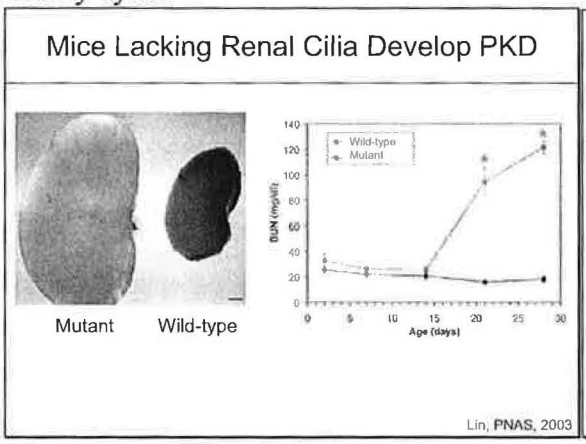


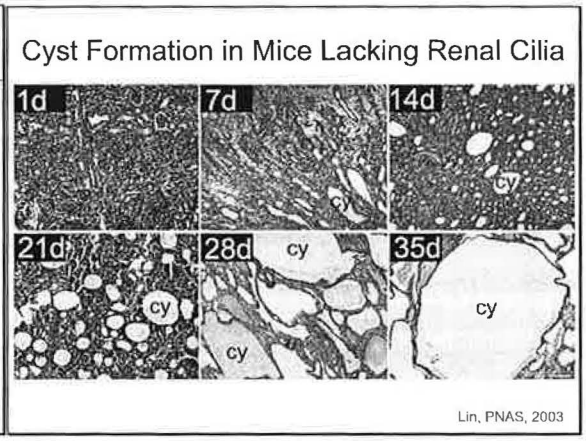
Primary cilia in the kidney are thought to function as mechanosensors of urine flow. Fluid flow over the surface of renal epithelial cells bends the primary cilia, and the magnitude of bending is related the velocity of the fluid flow (45). Moreover, bending of the primary cilium produces an increase in intracellular calcium concentration (37). Based on these findings it is thought that primary cilia in the kidney sense urine flow and transduce this signal as an increase in intracellular calcium. In other tissues cilia are also thought to have a sensory function. For



example, cilia in the retina are involved in light perception; cilia on olfactory cells are involved in detecting odors; primary cilia on neurons are involved in detecting neurotransmitters; and cilia in cartilage and bone may be involved in sensing mechanical strain. These results suggest that primary cilia function as antenna detecting the cell's environment (48).

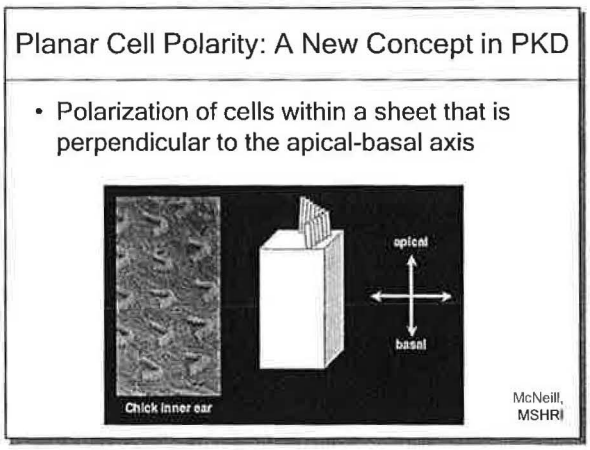
Three lines of evidence suggest that PKD arises from abnormalities in the primary cilium. First, polycystin-1 and polycystin-2 are located in primary cilia (59). Second, *Pkd1* and *Pkd2* mutant cells produce primary cilia but fail to increase intracellular calcium in response to fluid flow (32). Third, mutant mice that lack renal cilia develop kidney cysts and renal failure similar to humans with PKD (27). Taken together, these results suggest that mutations of polycystin-1 or polycystin-2 or the loss of primary cilia disrupt flow-dependent ciliary signaling and produce kidney cysts.



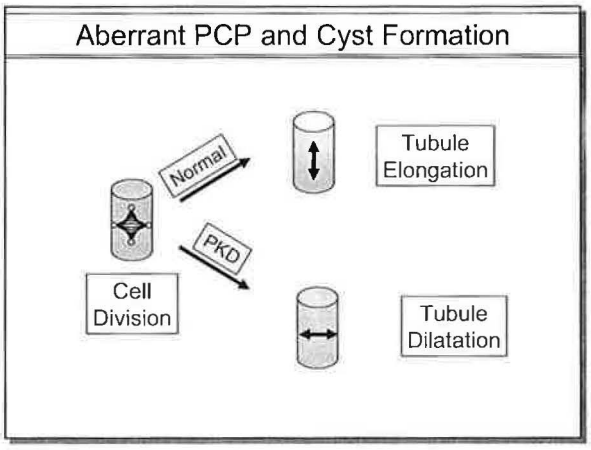


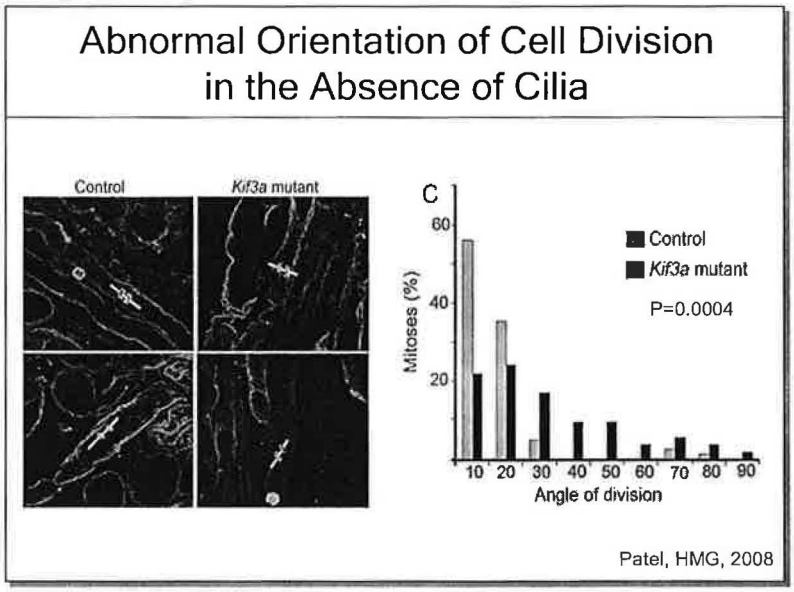
Planar Cell Polarity

Another new concept in the pathogenesis of PKD is planar cell polarity (PCP). PCP refers to the polarization of cells within a plane that is perpendicular to the apical-basal axis. We are most familiar with apical-basal polarity in which epithelial cells in the kidney have distinct proteins in their apical and basolateral membranes. PCP refers to polarity along an axis that is perpendicular to the apical-basal axis (24). The clearest example of PCP is in the inner ear where cells in the cochlea contain hairs that are arrayed in a specific pattern on their apical surface. The hair cells form a V-shaped structure in which the tip of the V always points medially. This orientation of hairs is a reflection of PCP and is essential for the function of the inner ear. Other examples of PCP can be found in the Drosophila wing in which the wing hairs always point towards the distal tip. PCP is essential for normal embryonic development. For example, elongation of the body axis during gastrulation involves a process called convergent-extension in which cells are reorganized in the plane of the epithelium. Similarly the development of the neural tube is also dependent on PCP. PCP is required for the orientation of cell division on the surface of the elongating embryo.



Defects in PCP were first identified in PKD by Dr. Marco Pontoglio's group at the Pasteur Institute (10). These investigators studied a rat model of ARPKD and detected abnormalities in PCP that were manifested as abnormalities in the orientation of cell division. In wild-type kidney tubules, the cells divided along an axis that was parallel to the longitudinal axis of the tubules. Consequently, cell division resulted in elongation of the tubule. In contrast, in the PCK rat model of ARPKD, the orientation of cell division was randomized. Cells no longer divided parallel to the axis of the tubule. If cells instead divided perpendicular to the axis of the tubules, dilatation of the tubule would result rather than elongation. Tubule dilatation is the first step in the formation of a kidney cyst. Recent studies from our laboratory have also found abnormalities of PCP in a mouse model of PKD that lacks renal cilia (35). Examination of pre-cystic tubules that lacked primary cilia showed randomization of the orientation of cell division. These results indicate that primary cilia are required for the maintenance of PCP in the kidney and that abnormalities of PCP produce kidney cysts. The mechanism by which the primary cilium regulates PCP is not known. In some tissues, PCP is dependent on non-canonical Wnt signaling. The loss of primary cilia has been shown to produce an imbalance between canonical and non-canonical Wnt signaling. Another possibility is that proteins shed by the primary cilium produce gradients that are required for the establishment of PCP (23). The role of primary cilia and PCP in the pathogenesis of PKD is currently being hotly investigated.





Treatment

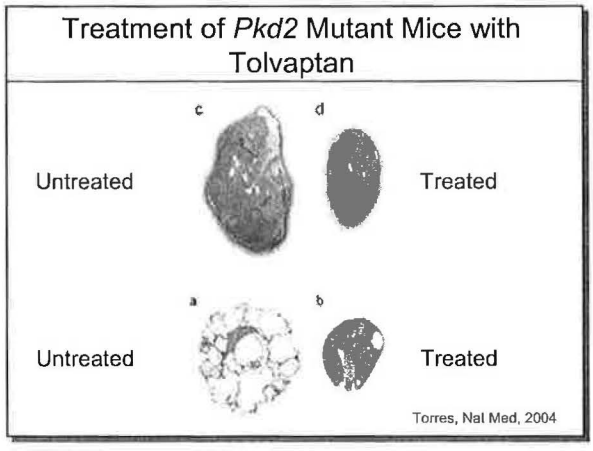
No cure or effective treatment of PKD currently exists. Definitive cure through gene therapy is unlikely to be available in the near future. Therefore, the current management of PKD consists of supportive measures (6), such as analgesics for pain, lipophilic antibiotics for cyst infection, antihypertensive drugs with the goal of keeping blood pressure less than 125/75, antibiotic prophylaxis for cardiac valvular disease, avoiding estrogen in women (increases the size of liver cysts), and avoiding caffeine (increases cyclic AMP, see below). The MDRD study showed that dietary protein restriction had no impact on the rate of decline of GFR in patients with PKD.

Although no specific treatment is currently available, several investigational drugs are currently in clinical trials in PKD. The goal of these therapies is to inhibit cell proliferation and correct the abnormalities in cell signaling that are arise from mutations in the polycystins. In many respects, PKD can be thought of as a neoplastic disorder characterized by dysregulated cell proliferation, apoptosis, cellular dedifferentiation, and fluid secretion. Numerous cell signaling pathways are inappropriately activated in PKD and could represent potential targets for therapy, including cyclic AMP, MAP kinase, JAK/STAT, Akt/mTOR, and Wnt/beta-catenin. Indeed, a major challenge is to understand which of these pathway alterations are important for cyst growth and which are secondary.

A recent advance has been the generation of orthologous animal models of human PKD. The genes that cause PKD in humans are highly evolutionarily conserved. Orthologous animal models carry mutations in the same genes as humans with PKD. These animal models, which were produced with gene targeting techniques, include *Pkd1*, *Pkd2*, and *Pkhd1* knockout mice that have mutations in the genes responsible for ADPKD and ARPKD (56). Since homozygous null *Pkd1* and *Pkd2* mutant mice are embryonic lethal, conditional *Pkd1* and *Pkd2* knockout mice have recently been generated in which the genes can be selectively inactivated in the kidney (46). This feature permits the animals to survive after birth and makes them amenable to therapeutic interventions. An orthologous rat model of ARPKD is represented by the PCK rat. The availability of orthologous animal models has greatly improved the evaluation of new compounds that may be beneficial in human PKD. Already, four drugs that reduce cyst formation in orthologous animal models have been identified: Tolvaptan (52), Octreotide (28), Pioglitazone (30), and the Chinese herb Triptolide (26). Two drugs, Rapamycin and Roscovitine, have shown benefits in non-orthologous animal models. Because these latter drugs were tested in non-orthologous models, their relevance to human PKD is less certain.

Tolvaptan

A common abnormality seen in different models of PKD is inappropriate activation of cAMP-dependent signaling (58, 50). Cyclic AMP is a second messenger that is produced by adenylyl cyclase, usually in response to the binding of hormones to G-protein coupled receptors (GPCRs). Normally, cAMP inhibits the growth of renal epithelial cells. However, in cyst epithelial cells, cAMP accumulates and stimulates cell growth, possibly because of activation of MAP kinases. One hormone that regulates cAMP levels in kidney cells is vasopressin (ADH). Vasopressin binds to the V_2 vasopressin receptor, activates adenylyl cyclase, and increases the levels of cyclic AMP. Increased cAMP promotes fluid secretion and cell proliferation in cyst epithelial cells. Increased levels of cyclic AMP have been observed in orthologous animal models of PKD including *Pkd2* mutant mice and PCK rats (52). A recent study provides additional evidence for the role of vasopressin in cyst

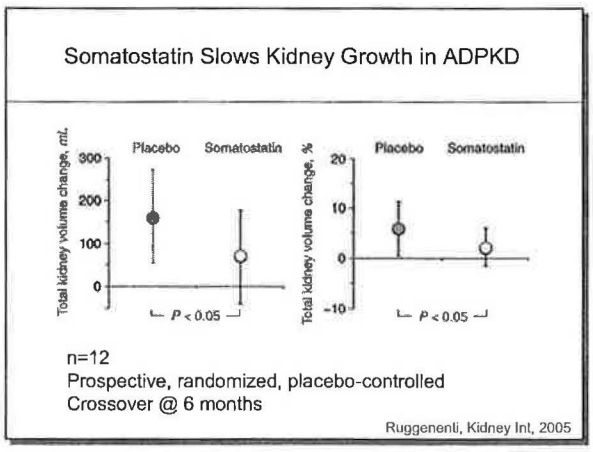


formation (54). In this study, PCK rats were crossed with the Brattleboro rat which is congenitally deficient in vasopressin. PCK rats that lack vasopressin showed considerably reduced cyst formation. The administration of exogenous vasopressin to these rats restored cyst formation providing strong evidence for the role of vasopressin in this model.

Tolvaptan is an investigational drug that acts by blocking the binding of vasopressin to the V_2 vasopressin receptor. Tolvaptan has been shown to decrease cAMP levels and inhibit cyst progression in two orthologous animal models of human PKD, the PCK rat and *Pkd2* mutant mice (52, 11). Based on these promising pre-clinical studies there is considerable interest in the use of Tolvaptan in human PKD. Fortunately, Tolvaptan was already in clinical trials for the treatment of edematous states such as CHF and cirrhosis, where it has been found to be safe and effective. Another advantage is that Tolvaptan can be given by the oral route. The FDA granted fast track approval for the use of Tolvaptan in ADPKD in 2006. A phase II clinical study of safety, dosing, and side affects has been completed, and a multi-center phase III study is underway (TEMPO3/4)(51, 6). The side effects of Tolvaptan are predictable based on its mechanism of action and include increased thirst and polyuria.

Why not just drink a lot of water?

Since vasopressin has deleterious effects in animal models of PKD, it has been suggested that another way to suppress vasopressin would be to drink large amounts of water. The release of vasopressin from the pituitary gland is regulated by osmolality, such that an increase in serum osmolality stimulates vasopressin release. Conversely, if serum osmolality is reduced, vasopressin release is decreased. Based on this rationale some nephrologists have proposed that patients with PKD should drink large amounts of water to reduce serum osmolality, suppress vasopressin release, and decrease cyclic AMP levels in the kidney. Indeed, one recent study showed that high water intake decreased cyst formation and reduced BUN levels in the PCK rat (31). On the other hand, a retrospective analysis of the MDRD study suggested that high urine volume and low urine osmolality might be risk factors for faster progression of PKD (19). Because of these conflicting results, more studies on high water intake are needed. At the current time, patients should be encouraged to drink water as needed to avoid dehydration and to avoid caffeinated beverages which increase cyclic AMP levels.



Octreotide

Octreotide is a long-acting analogue of the hormone somatostatin. Octreotide binds to cell surface somatostatin receptors and decreases intracellular cAMP levels and inhibits fluid secretion (13). Administration of octreotide to PCK rats reduced cAMP levels in bile duct cells and decreased the formation of cysts in the liver and kidney, although there was no improvement in renal function (28). A small prospective randomized placebo-controlled study with crossover at 6 months has been performed in 12

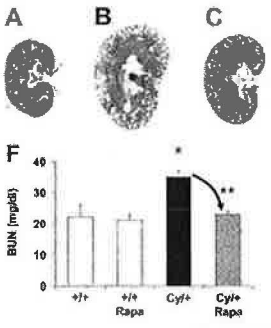
individuals with PKD (42). There was a significant decrease in total kidney volume in the octreotide-treated group compared to the placebo-treated group. Since decreased kidney volume may reflect a lower risk of progression of PKD, this study suggests that the use of somatostatin may be beneficial. Two phase III clinical trials are underway, one at the Amrio Negri Hospital with three year follow-up and another study on polycystic liver disease being conducted at the Mayo Clinic. The side effects include diarrhea, hyperglycemia, and liver function abnormalities.

Rapamycin

Rapamycin is an immunosuppressive drug that inhibits mTOR (mammalian target of rapamycin). Activation of mTOR has been detected in the cells lining the cysts in human polycystic kidneys (47). Polycystin-1 has been shown to interact with tuberin (TSC2), a component of the tuberous sclerosis complex (25, 29). TSC2 and TSC1 inhibit Rheb, which activates mTOR. Under normal conditions, polycystin-1 appears to inhibit mTOR. In the absence of polycystin-1, the inhibition of Rheb is relieved resulting in activation of mTOR, which promotes protein translation and cell growth. Rapamycin acts by inhibiting mTOR and has been shown to be beneficial in a non-orthologous rat model of PKD (49). Administration of rapamycin to Han:SPRD rats reduced cyst formation and decreased BUN levels.

In a widely publicized study published in PNAS in 2006, Thomas Weimbs' group suggested that rapamycin might also be beneficial in human PKD (47). They examined a small group of PKD patients who had undergone kidney transplantation without removal of their native polycystic kidneys and were treated with immunosuppressive regimens that either contained rapamycin or did not contain rapamycin. In four patients who received rapamycin, the volume of the native kidneys decreased by 24% compared to 8.6% in three patients treated with non-rapamycin protocols. These results suggested that rapamycin may reduce the size of polycystic kidneys. However, the results were not reproduced in an independent study from the Mayo Clinic (38). Treatment with rapamycin reduced liver size but did not have a significant effect on kidney size. Rapamycin is currently used for immunosuppression to prevent transplant rejection and is not approved for use in PKD. Moreover, rapamycin has never been tested in an orthologous animal model of PKD, which should be a prerequisite before a clinical study is conducted in humans. Rapamycin has significant side effects including infection and lymphoma, which may preclude its long-term use in a chronic disease such as PKD. Larger clinical trials are needed to determine whether rapamycin, everolimus, or other mTOR inhibitors are effective in PKD.

Treatment of Murine PKD with Rapamycin

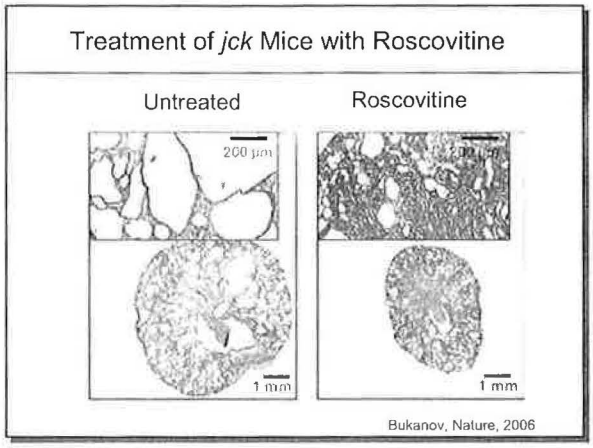


Tao, JASN, 2005

Rapamycin Reduces Native Kidney Size in Transplant Recipients with PKD

Immunosuppression (n)	Rapamycin (4)	Non-rapamycin (3)
Follow-up	24 months	40 months
% kidney volume change	–24.8% ± 9.7% (P =.001)	–8.6% ± 11.2% (P = 0.16)
% kidney volume change/month	–1.4% ± 0.8% (P =.005)	–0.3% ± 0.4% (P = 0.22)

Shillingford, PNAS, 2006



Roscovitine

The newest drug that has appeared on the scene for possible use in the treatment of PKD is roscovitine. Roscovitine is a selective inhibitor of cyclin-dependent kinases (CDK), which regulate cell cycle progression. In preclinical studies roscovitine has been shown to reduce the cyst formation in the non-orthologous jck mouse model of PKD (5). One unique aspect of this study was the observation that roscovitine did not need to be given continuously and that intermittent dosing was also effective. Moreover, the beneficial effects appeared to be long-lasting. These are promising results for the treatment of a chronic disease in which life-long therapy may be required. Roscovitine is currently in phase I and phase II clinical trials in cancer. Side effects are worrisome and include acute renal failure. Studies in an orthologous animal model are needed before studies are performed in human PKD.

Summary

In summary, PKD is a common genetic cause of kidney failure in humans. PKD is a systemic disorder with significant renal and cardiovascular manifestations. PKD is caused by mutations in *PKD1* or *PKD2*. PKD is a ciliopathy that may arise from defects in planar cell polarity. The diagnosis of PKD is established by renal ultrasound. Genetic testing is available and may be more commonplace with the enactment of GINA. The CRISP study has shown that larger kidney volume predicts a more rapid decline in GFR. ACE inhibitors and ARBs have not been proven to improve renal survival or mortality, but an ongoing NIH study is trying to clarify this point. Promising new drugs are in clinical trials including tolvaptan, rapamycin, and octreotide.

Finally, patients with PKD and their relatives and physicians should be made aware of the PKD Foundation, a non-profit organization that supports research and education in polycystic kidney disease. The Foundation has a website www.pkdcure.org that contains the latest information on PKD research and clinical trials. This year, the annual convention of the PKD Foundation will be held in Dallas on June 20–22. Patients and their families are encouraged to attend. More information about registration for this meeting can be found on the Foundation website.

Acknowledgments

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