Preface

> "Rule IV. There is need of a method for finding out the truth.
> Rule V. Method consists entirely in the order and disposition of the objects toward which our mental vision must be directed if we would find out any truth. We shall comply with it exactly if we reduce involved and obscure propositions step be step to those that are simpler, and then starting with the intuitive apprehension of all those that are absolutely simple, attempt to ascend to the knowledge of all others by precisely similar steps."
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> —Rene Descartes, *Rules for the Direction of Mind*

> "…Perhaps he would sooner satisfy himself by resolving light into colours as far as may be done by Art, and then by examining the properties of those colours apart, and afterwards by trying the effects of reconjoyning two or more or all of those, and lastly by separating them again to examine what changes that reconjunction had wrought in them. This will prove a tedious and difficult task to do it as it ought to be done but I could not be satisfied till I had gone through it."
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> —From Newton's letter, quoted in *The Life of Isaac Newton* by Richard Westfall. Cambridge University Press, 1993.

As much as the progress of a discipline depends on the progress of methods it uses, the overall goal of *Renal Disease: Techniques and Protocols* is to provide a comprehensive and balanced account of adequacy, advantages, and potential pitfalls of various modern approaches to study renal function in health and disease. Toward this end, any possible hesitation in selecting the shortest, safest, and most picturesque path to one's research summit should be alleviated by the expert contributors, who have already taken a similar road and are keen to share their observations. It is our sincere hope that this collection of technical approaches should become a *vade mecum*, which will be both a user-friendly guide for the uninitiated and a thoughtful counselor for the experienced scholar of fluid–electrolyte homeostasis and kidney function.

The last few years have witnessed the completion of the human genome project, development of high-throughput techniques for the screening of expressed genes, and the emergence of technological platforms for the next major enterprise—proteomics research. Yet, the basic tenets of approaching the problem at hand have not undergone transformation since the time when

Rene Descartes formulated them. Simplified models, devoid of the complexities and "obscurities" of reality, remain the bedrock of investigation. Lessons learned are further tested in more complex models, ascending ultimately to the organismal level. Therefore, the flow of chapters in this book has been designed to reflect upon this process—from simple models to integrative physiology.

With this in mind, *Renal Disease: Techniques and Protocols* is subdivided into five sections: (I) Optimizing the Usage of Models of Renal Disease, (II) Choices of Imaging Techniques, (III) Studies of Embryonic Development of the Kidney, (IV) Approaches to Study Molecular Mechanisms of Disease, and (V) Technical Means to Assess Functional Correlates of Disease. Though intricately interconnected, such a subdivision should provide an investigator with a possible path to follow in the course of investigation.

Certain technological areas are not represented in this volume (i.e., gene therapy). This does not reflect this editor's negligence, but rather acknowledges that it has become a subject for another volume published in this series and the interested reader is referred to that edition ***(1)***.

Methods per se are not science, but mere tools to achieve scientific goals. And yet in this process new techniques are being born or the old ones modified to satisfy the precise goals of a researcher. The intelligent use of the technological armamentarium is a valuable assistant in our inquiries. It is for this reason that philosophers and thinkers of all times have developed a body of literature that summarizes the diversity of scientific approaches. In addition to the reductive and inductive methods, illustrated by the above quotations from Descartes and Newton, approaches to a problem that are based on theoretical predictions initially unsupported by the facts ("dogmatic method"), reliance on a chance discovery ("haphazard experiment"), as well as the "method of contradiction" and the "method of recodification," searching for known patterns in unknown situations (or vice versa), all enrich the repertoire of strategies to be selected by an investigator ***(2)***. The advent of high-throughput screening technologies provides, at least at the first glance, a typical example of a shifting paradigm of research strategies. In contrast to the "dogmatic method," these screening approaches are, basically, unbiased and unenlightened by a hypothesis—perfect examples of what one would call "a fishing expedition." When successful however, they offer the researcher a previously concealed and entirely unexpected set of data. These in turn require the engagement of a "reductive method" to try sorting out potential pathways that have led to the fact(s) disclosed in an unbiased fashion and their consequences. Thus, starting with the Newtonian stance of *hypotheses non fingo* (I don't make hypotheses), these high throughput approaches require just the opposite at the stage when the output from the technological platforms reaches the desk of an astonished investigator.

With more than 50,000 scientific periodicals published worldwide that in toto print weekly more than 40,000 scientific articles, the level of informational barrage to which an investigator is exposed has become almost unbearable. No matter which strategic decisions for attacking the problem at hand have been made, the next challenge of selecting the correct tool set confronts every investigator. Under the stress of informational overflow, this selection turns into an overly complex process. Therefore, a manual, guiding investigators among the thicket of available techniques and providing them with expert insights into the advantages and bottlenecks of each, should serve to strengthen the scientific backbone and save time. With these goals in mind, we offer the reader *Renal Disease: Techniques and Protocols*.

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References

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2

Models of Polycystic Kidney Disease

Poornima Upadhya

1. Introduction

Polycystic kidney disease (PKD) is a potentially life-threatening disorder that affects both adult and pediatric patients. PKD can be either inherited as a dominant (ADPKD) or a recessive trait (ARPKD) or acquired. The disease is characterized by massive renal enlargement associated with the growth of fluid-filled intrarenal cysts. ADPKD, the most common cystic disease, is caused by mutations at three distinct loci: *PKD1*, *PKD2*, and *PKD3*. The *PKD1* locus was mapped to human Chr 16p13.3, and the *PKD2* locus was mapped to human Chr 4q21–23. The *PKD3* locus has not yet been mapped. *PKD1* is the most commonly inherited mutation. Patients with ADPKD develop renal, hepatic, and pancreatic cysts, abdominal and inguinal hernias, heart-valve defects, and aortic and cerebral aneurysms ***(1)***. ARPKD is encountered less frequently. *PKHD1*, a locus on human Chr 6p21-cen that predisposes individuals to develop ARPKD, has been reported. ARPKD patients primarily develop cysts in the collecting ducts, with hepatic fibrosis as an associated extrarenal manifestation ***(2)***.

Genetic studies have identified the normal products of the *PKD1*, *PKD2*, and *PHKD1* loci ***(3–5)***. Efforts are underway to decipher the functions of the normal protein encoded by each of these three loci. However, the detailed understanding of cystogenesis caused by ADPKD and ARPKD is complicated by their variability with respect to age of onset and extra-renal manifestations ***(6,7)***. This variation suggests that other genes modulate the clinical manifestation of PKD caused by any of the previously identified *PKD1*, *PKD2*, *PKD3*, or *PKHD1* disease loci. Because of the uncontrollable genetic variability between human patients, identification of the genes that modulate disease severity in the human population is currently an insuperable problem. Animal models on defined genetic backgrounds substantially simplify the identification of these modifying factors.

From: *Methods in Molecular Medicine, vol. 86: Renal Disease: Techniques and Protocols*
Edited by: M. S. Goligorsky © Humana Press Inc., Totowa, NJ

There are a number of animal models for PKD ***(8,9)***. In some of the models, the severity of the disease resulting from the main mutation varies with the genetic background of the mouse ***(10–12)***. Characterization studies in the various animal models of PKD will help us gain a fuller comprehension of the clinical manifestations of the disease in humans. This chapter reviews the characteristic morphological features and biochemical and molecular alterations in the common rodent models for PKD.

2. Inherited Models of PKD

2.1. cpk *Mouse*

The *cpk* mutation on mouse Chr 12 arose spontaneously in the C57BL/6J strain ***(13)***. PKD in *cpk* homozygotes is aggressive, and its rapid progression to terminal stages leads to death at approx 3 wk of age. Although renal abnormalities are limited to the homozygous *cpk* animals, hepatic cysts have been reported in older heterozygotes. On the DBA/2J background, in addition to the renal phenotype, the *cpk* mutation produces pancreatic and hepatic fibrosis and dilation, making this an attractive animal model for the study of human ARPKD. Light and transmission electron microscopy studies have shown that renal abnormalities appear in the earliest stages within the proximal tubules. However, the site of involvement shifts to cortical and collecting ducts as the disease progresses. Death is most probably the result of end-stage renal failure, since blood-urea nitrogen and serum creatinine levels are elevated at approx 3 wk of age ***(14)***.

Biochemical and molecular studies have led to the identification of several cellular and extracellular matrix (ECM) abnormalities in the *cpk* model. Enhanced expression of the proto-oncogenes such as c-myc, c-fos, and c-Ki-ras in the homozygous *cpk/cpk* mutants may reflect epithelial hyperplasia. In addition, the increased level of epidermal growth factor (EGF) in the renal cystic fluid, coupled with the apical mis-localization of the EGF receptor (EGFR), is believed to have mitogenic effects on the cystic epithelium. The epithelial cells of *cpk* homozygotes also show some features of dedifferentiation. Although apical and basolateral localization of Na^+-K^+ATPase expression is a normal transient feature during early renal tubule development, the pump is restricted to the basolateral side later in terminally differentiated cells. However, in some of the cystic collecting tubules the apical membrane expression of Na^+-K^+ATPase remains significantly increased, suggesting the loss of differentiated phenotype. Further evidence for the loss of differentiation comes from the presence of abnormally high levels of sulphated glycoprotein. This suggests that PKD in the *cpk* mice may be caused in part by defective terminal differentiation of the tubular epithelial cells. Additional abnormalities reported in the

cpk kidneys include increased expression of basement-membrane constituents and remodeling enzymes, matrix metalloproteinases (MMPs), and their specific tissue inhibitors, TIMPs.

Recently, a novel gene, *cystin*, which is disrupted in *cpk* mice was cloned by positional cloning. When expressed exogenously in polarized renal epithelial cells, cystin was detected in cilia, and its expression overlapped with *polaris*, another PKD-related protein. Cystin expression appears to be enriched in the ciliary axoneme. This has led to the speculation that the cystin bound to the axonemal membrane functions as part of a molecular scaffold that stabilizes microtubule assembly within the ciliary axoneme ***(14)***. This speculation is supported by the observation of Woo et al. that weekly injections of taxol to the *cpk* mice was able to prolong the survival of the *cpk* mice to more than 200 d with a remarkable reduction in the number of cysts in the taxol-treated animals ***(16)***.

2.2. pcy *Mouse*

Takahashi et al. reported a spontaneous occurrence of a recessive form of PKD in the KK strain of the diabetic mouse. Genetic linkage analysis showed that the *pcy* mutation is located on mouse Chr 9 ***(17)***. Kidney malformation and progression of PKD in inbred DBA/2-*pcy/pcy* mice has been characterized in detail. Renal cysts develop in all segments of the nephron, and progressively enlarge with age eventually severely distorting the entire kidney in adult animals. Hallmark features of cystic changes similar to those in human polycystic kidney epithelia such as renal tubular apoptosis, cellular hyperplasia, and abnormal basement membrane were observed. However, unlike the apical mislocalization of the Na^+-K^+ATPase in the cystic epithelial cells from some of the human ADPKD patients and the *cpk* mouse, only basolateral localization of the sodium pump was identified in *pcy* renal tubular epithelial cells. The mRNA levels encoding for growth-related proteins, such as TGF-β, PDGF-α, PDGF-β, IGF-1, basic FGF, and cyclin mRNA showed progressive increase with the age of animals. However, since the changes in expression of these genes did not take place in the earliest stages of the renal disease, but followed the progression of the disease, it is unlikely that they are key initiating players of renal cyst development ***(18,19)***.

2.3. bpk/jcpk *Mouse*

The *bpk* mutation arose spontaneously in the inbred Balb/c strain. The mutation is autosomal recessive and maps to mouse Chr 10. Cysts develop predominantly in proximal tubules of the kidney in the earliest stages of PKD, and homozygous mutants die at about 1 mo of age. Another mutation, *jcpk* was induced by chlorambucil mutagenesis. The renal disease caused by this mutation is extremely aggressive, and homozygotes survive for less than 2 wk. Cysts

appear in all segments of the nephron, including the glomerulus. Because the *bpk* and *jcpk* mutations were mapped close to each other on Chr 10, a complementation test was performed. The complementation test demonstrated that these two mutations were allelic. This was surprising, since despite their distinctly different PKD phenotypes, the *bpk* and *jcpk* mutations appeared to disrupt the same gene. However, since *jcpk* is a chlorambucil mutation that generally involves large deletions or chromosomal rearrangements, it is likely that *jcpk* involves a significantly larger genomic alteration involving two closely linked genes, one of which is *bpk*. These two possibilities can be distinguished once the molecular nature of mutations is determined ***(20)***.

2.4. kat *Mouse*

The *kat* mutation arose spontaneously on the RBF/Dn background. The mutation is autosomal recessive, and maps to mouse Chr 8 ***(21)***. Cysts appear in all segments of the nephron, including the glomerulus. The homozygous mutant mice exhibit a latent-onset slowly progressing form of PKD with renal pathology similar to human ADPKD. In addition, the *kat* mutation causes pleiotropic effects that include facial dysmorphism, dwarfism, male sterility, anemia, and cystic choroid plexus ***(22)***. The gene altered by the *kat* mutation was cloned by positional cloning. The gene altered is *Nek1* (NIMA-related kinase-1) that encodes for a dual-specificity protein kinase. The kinase domain is most similar to NIMA, a protein kinase that controls initiation of mitosis in *Aspergillus nidulans*. The complex pleiotropic phenotypes seen in the homozygous mutant animals suggest that the NEK1 protein participates in different signaling pathways to regulate diverse cellular processes. It has been hypothesized that in the kidney, NEK1 protein belongs to a signaling pathway that promotes the full maturation of renal tubular epithelial cells, suggesting that the loss of NEK1 function traps these tubular epithelial cells in a state of permanent immaturity and growth ***(23)***.

During the mapping studies, it was noted that the genetic background or modifier genes alter the severity of PKD caused by the mutation. Genome scans using molecular markers revealed three modifier loci that affect the severity of the PKD caused by the mutation. Additional modifier loci that interact with and modulate the effects of these three modifier loci were also identified. The mapping of these modifier genes, and their eventual identification, will help to reveal factors that can delay disease progression ***(12)***.

2.5. jck *Mouse*

The *jck* mutation arose spontaneously in the Tg.ple transgenic line and did not segregate with the transgene. This mouse mutation is inherited as an autosomal recessive trait, and maps to Chr 11. The progression of PKD in this

mouse model is slower than that seen in the *cpk* model. Cystic kidneys are detected by 6 wk, and animals survive until 20–25 wk of age. Histological analysis shows cysts predominating in the outer medulla and cortex. Very little is known about the cascade of events that leads to the clinical manifestation of the disease in this model and the role of growth hormones, ECM alterations, proto-oncogene expression, cell proliferation and differentiation ***(11,24)***.

2.6. cy *Rat*

The *cy* mutation occurred spontaneously in Han:SPRD rats. Genetic analysis showed that the mutation was inherited as a dominant trait. Heterozygous rats develop a slowly progressing cystic disease that is accompanied with interstitial fibrosis and thickened basement membrane. In this animal model there are extrarenal manifestations besides PKD; these include hyperparathyroidism, osteodystrophia fibrosa, and metastatic calcification of the lungs, stomach, and heart. Histological analysis of the cystic kidney showed gender dimorphism that was more pronounced than in humans: females survive considerably longer than males. Taking the gender difference into account, Zeier et al. tested the impact of castration on progression. A significant slowing of progression occurred in the castrated rats, although the serum urea concentrations were still higher than usually seen in the female rats ***(25)***. Similar beneficial effects were also seen in male mutants treated with methylprednisolone, which could reduce the interstitial inflammation and fibrosis, a common feature of PKD. However, mutant female animals did not respond to methylprednisolone treatment. The Han:SPRD cy/+ rat represents a well-documented rat model of ADPKD, with a number of features that resemble the human disease. Thus, this model has been extensively used for studying the pathophysiological events and evaluation of therapeutic interventions ***(26)***.

2.7. pck *Rat*

The *pck* rat is a recently identified model of PKD that developed spontaneously in the rat strain Crj:CD/SD ***(27)***. The *pck* mutation is inherited as an autosomal recessive trait. The *pck* rats develop liver cysts and progressive cystic enlargement of the kidneys after the first week of life. The renal cysts develop as focal process from thick ascending loops of Henle, distal tubules, and collecting ducts in the corticomedullary and outer medulla region. Apoptosis is common, and affects normal as well as dilated tubules. The basement membranes of the cyst walls exhibited a variety of alterations, including thinning, lamellation, and thickening. Segmental glomerulosclerosis, focal interstitial fibrosis, and inflammation are evident in 2-mo old mutant animals. The PKD is more severe in male than in female *pck* rats, as reflected

by the higher kidney weights, although there is no gender difference in the severity of the cystic liver disease.

This *pck* rat is a valuable animal model of ARPKD. Recent genetic analysis of the ARPKD region in humans, identified a candidate gene, *PKHD1*. The *pck* mutation was found to be a splicing defect in the rat ortholog of this candidate gene. The *PKHD1* gene is predicted to encode a large novel protein, fibrocystin, with multiple copies of a domain shared with plexins and transcription factors. Based on its structural features, fibrocystin is believed to be a receptor protein that acts in collecting-duct and biliary differentiation. Interestingly, although the same gene is mutated in both the *pck* rat model and the human ARPKD patients, the *pck* rat model shows some phenotypic differences from the human disease, including the degree of hepatic cyst development, predominant development of renal cysts in the outer medullary-collecting ducts, and mild portal fibrosis in the liver, without formation of fibrous septa or development of portal hypertension ***(5)***. This suggests that molecular nature of the mutation at the *PKHD1* locus may partially account for phenotypic variability seen among ARPKD patients.

3. Transgenic Models of PKD

3.1. orpk *Mouse*

The *orpk* transgenic line was developed as a part of a large-scale insertional mutagenesis program. The mutation maps to mouse Chr 14 and is inherited as a recessive trait. The homozygous mutant animals on the FVB/N inbred background have pre-axial polydactyly on all limbs, PKD, and abnormalities of the intrahepatic biliary tract, and are severely growth-retarded. Most of the *orpk*-mutant mice on the FVB/N inbred background die during the first week of life. However, on the C3H inbred genetic background, the mutant mice live longer, have polydactyly that is more variable, develop renal cysts at a slower rate, and have a less aggressive liver lesion. In the kidney, large cysts form in the collecting tubules, yet in the liver there is a consistent biliary hyperplasia and bile ductule ectasia, along with portal fibrosis. These lesions in the kidney and liver are remarkably similar to those seen in human ARPKD. Like human patients with ADPKD or ARPKD, the homozygous mutants exhibit increased expression and apical mis-localization of EGFR. These changes in EGFR were shown to be of physiological relevance, since genetic or pharmacological inhibition of EGFR activity results in a significant improvement in the renal pathology and function ***(28,29)***.

Using the integrated transgene as a molecular marker, the mutated gene was cloned. In both multi- and mono-ciliated epithelium and in sperm, polaris, the protein encoded by the mutated gene was localized to the basal bodies and in

the axoneme. A cortical collecting duct cell line has been derived from *orpk* mice. These cells were found to be devoid of cilia, but the defect could be corrected by re-expression of the wild-type polaris gene. These data suggest that the primary cilia are important for normal renal function and/or development and that the ciliary defect may be a contributing factor to the cystic disease in *orpk* mice. Further characterization of these cells will be important in elucidating the physiological role of renal cilia and their relationship to cystic disease ***(30)***.

3.2. SBM *Mouse*

The *SBM* transgenic mice carry a fusion gene that includes the SV40 enhancer, the β-globin promoter, and the c-myc coding region, which is expressed at high levels in the renal tubular epithelium. These transgenic mice develop markedly enlarged kidneys, runting, and muscular atrophy. The cysts are scattered throughout the cortex and medulla, and the transgenic animals die from renal failure by 5 mo of age. In a large number of SBM transgenic mice, the kidneys contain focal interstitial aggregates of atypical plasma cells. The specific elevated expression of c-myc in hyperplastic renal tubular cyst epithelium of the *SBM* and *cpk* mice, suggests that cyst formation can arise through the deregulation of tubular epithelial cell proliferation ***(31)***.

3.3. bcl-*2–/– Knockout*

bcl-2 is distinguished from other proto-oncogenes by its death-repressor activity and intracellular localization. Apoptosis is known to occur in both the nephrogenic and medullary region of the developing kidney, and follows a distinct developmental time-course. The Bcl-2 protein is expressed in the developing human and murine kidney. *bcl-2–/–* mice complete embryonic development, but display growth retardation and early mortality. Veis et al. found that hematopoiesis—including lymphocyte differentiation—was initially normal, but the thymus and spleen underwent massive apoptotic involution. The early mortality of the mutants was caused by renal failure resulting from a severe PKD. The presence of dilated proximal and distal tubular segments and hyperproliferation of epithelium and interstitium characterized the renal cystic disease ***(32)***. Cystic kidneys from *bcl-2–/–* mice displayed nuclear localization of β-catenin and a loss of apical brush-border actin staining. However, the protein levels of α-catenin, β-catenin, actin, and E-cadherin were not altered in cystic kidneys compared with normal kidneys. Recently, the expression and activity of focal adhesion tyrosine phosphatases Src homology-2 domain phosphatase (SHP-2), protein tyrosine phosphatase (PTP 1B), and PTP-proline, glutamate, serine, and threonine sequences (PEST) during normal nephrogenesis and in cystic kidneys from *bcl-2–/–* mice were examined. Cystic kid-

neys from *bcl-2–/–* mice demonstrated a reduced activity, expression, and altered distribution of SHP-2 and PTP 1B. The altered regulation of PTP 1B and SHP-2 in kidneys from *bcl-2–/–* mice correlated with sustained phosphorylation of FAK and paxillin. Taken together, the renal cyst formation in the *bcl-2–/–* mice is believed to be a result of improper cell–cell interactions that interferes with renal maturation by continued activation of growth processes, including activation of FAK and paxillin ***(33)***.

4. Transgenic Models Involving Polycystins

Polycystins are a family of transmembrane proteins. As mentioned earlier, two of the polycystin family members, polycystin-1 and -2, are mutated in human ADPKD patients. Polycystin-1 is a 4302 amino acid (aa) glycoprotein. Important features of polycystin-1 include several transmembrane segments and a cytoplasmic C-terminal domain containing potential phosphorylation sites. Polycystin-2 is a 968-aa protein, with a predicted structure that includes two intracellular domains flanking six transmembrane segments. The protein has homology to the voltage-activated Ca^{2+} channel a_{1E} and Na^{+} voltage-dependent channels, as well as to the trp family of Ca^{2+} channels. In addition, there is a 29-aa EF hand motif involved in Ca^{2+} binding in the intracellular, C-terminal portion of the protein. The C-terminal tail of polycystin-1 interacts with that of polycystin-2, resulting in the formation of calcium-permeable nonselective cation channels in vitro, suggesting that extracellular signals can be transduced by the polycystin complex to regulate diverse cellular processes. Indeed, the cytoplasmic tail of polycystin-1 has been shown to signal via the G proteins, and its signaling pathway was shown to intersect with that of Wnts, a family of secreted signaling molecules. Polycystin-2 alone also mediates cation currents and functions as a Ca^{2+}-permeable nonselective cation channel ***(34,35)***. Both proteins are expressed during renal development, but their exact role in cyst formation and in other disease manifestation is unclear.

4.1. Pkd1-*Targeted Knockout*

None of the genetic animal models of PKD map to the murine *Pkd1* locus. In order to decipher the normal function of polycystin-1, Zhou et al. first introduced into mice by homologous recombination a *Pkd1* truncation mutation. The homozygous mutant mice carrying a deletion of exon 34 ($Pkd1^{del34}$) of *Pkd1* developed a severe PKD and pancreatic disease and died during the perinatal period ***(36)***.

Since the $Pkd1^{del34}$ mutation is a truncation mutation, it was not clear whether the phenotype of the animals carrying the mutant allele was the result of the altered function of the truncated form of polycystin-1 or caused by haploinsufficiency. In order to address this, Zhou et al. generated, by homolo-

gous recombination, a second targeted mouse mutant with a null mutation (*Pkd1*$^{-}$) in *Pkd1*. The null homozygotes (*Pkd1*$^{-/-}$) developed more aggressive but similar renal and cystic disease as the *Pkd1*$^{del34/del34}$ homozygotes. It was also reported that both the *Pkd1*$^{-/-}$ and *Pkd1*$^{del34/del34}$ homozygotes developed polyhydramnios, hydrops fetalis, occult spina bifida, and osteochondrodysplasia ***(36)***. Interestingly, homozygous mutants with another mutant allele, *Pkd1*^{L}, which produces mutant polycystin-1 protein that is 478 aa longer than that encoded by the *Pkd1*^{del34} mutant allele, show a more severe phenotype, as judged by embryonic lethality at E15.5, and also display a major vascular phenotype ***(37)***. Studies of these various knockouts have shown that normal polycystin-1 is required for maintaining the structural integrity of the vasculature and in epithelial and chondrocyte development. The studies also suggest that the molecular nature of mutation at the PKD1 locus may partly account for the phenotypic variability seen in ADPKD ***(37)***.

Interestingly, heterozygous *Pkd1*$^{+/del34}$ mice progressively developed scattered renal and hepatic cysts. Cysts were seen from the cortex to the inner medulla. Glomerular cysts were common. Cysts were often surrounded by atrophic parenchyma with interstitial fibrosis and inflammation. EGFR was mislocalized to apical membranes in cysts and some slightly dilated tubules, suggesting that EGFR mis-localization may serve as an early marker of cystic transformation in polycystin-1 deficiency. Liver cysts were filled with clear or dark-brown fluid, which represents the bile salt-independent fraction of bile, indicating that the cyst epithelia, although originating from biliary ductule epithelia, had altered secretory function. This is similar to the human condition, and supports the hypothesis that fluid accumulation in the cysts is primarily the result of increased secretion from the cystic epithelia. The prominent liver changes, combined with the absence of liver cysts in perinatal homozygotes, suggest that polycystin-1 is required in the maintenance—but not the formation—of biliary ducts. The gradual recruitment of cysts and the absence of polycystin-1 in some renal cysts in the *Pkd1*$^{+/del34}$ heterozygous animals are consistent with clinical progression in man ***(38)***. It is also consistent with the "two hit" model for cyst development. According to this theory, the germline mutation is insufficient to initiate cyst formation; however, if the wild-type allele required to generate the normal protein is altered by somatic mutation, then the affected cell initiates the cyst phenotype ***(39)***.

4.2. Pkd1 *Transgenic Mice*

Two transgenic lines, each with 30 copies of a 108-kb human genomic fragment containing the entire *Pkd1* gene plus the tuberous sclerosis gene, have been established. Transgenic animals often show hepatic cysts, bile-duct proliferation, and renal cystic phenotype, with multiple cysts that are mainly of

glomerular origin. Both transgenic lines were found to rescue the embryonic lethal phenotype of the homozygous *Pkd1*$^{del34/del34}$ animals, demonstrating that the human polycystin-1 can complement for the loss of the endogenous murine protein. The rescued animals were viable into adulthood, although more than one-half of them developed hepatic cystic disease in later life. Studies from this transgenic model of PKD suggest that the level of polycystin-1 may be an important parameter in regulating renal cyst formation ***(40)***.

4.3. Pkd2*-Targeted Knockout*

Wu et al., using embryonic stem-cell technology, introduced mutant exon 1 in tandem with the wild-type exon 1 at the mouse *Pkd2* locus. This resulted in an unstable allele (*Pkd2*^{WS25}) that underwent somatic inactivation by intragenic homologous recombination to produce a true null allele. Mice that were heterozygous (*Pkd2*$^{+/WS25}$) and homozygous (*Pkd2*$^{WS25/WS25}$) for this mutation developed polycystic kidney and liver lesions that were indistinguishable from human ADPKD. However, the kidneys from the *Pkd2*$^{WS25/WS25}$ mice showed a more severe but considerably heterogeneous renal phenotype when compared to the kidneys from the *Pkd2*$^{+/WS25}$ mice. Renal cysts arose from renal tubular cells that lost the capacity to produce Pkd2 protein. Somatic loss of *Pkd2* expression in heterozygous animals was both necessary and sufficient for renal cyst formation, suggesting a cellular recessive mechanism of cyst formation. Wu et al. also introduced a true null (*Pkd2*$^{-}$) mutation. The *Pkd2*$^{-/-}$ mice, which died in utero between embryonic d E 13.5 and parturition, had structural defects in cardiac septation and cyst formation in maturing nephrons and pancreatic ducts. Despite the absence of cystic disease, the adult *Pkd2*$^{+/-}$ mice had a shorter lifespan compared to their wild-type litter-mates, suggesting the deleterious effect of polycystin-2 haploinsufficiency on long-term survival. These two models have shown that in addition to the role of polycystin-2 in maintenance of renal function, the protein is also essential for the normal development of the interventricular and interatrial septa and the pancreatic duct ***(41)***.

5. Molecular Mechanisms of PKD

PKD has puzzled the scientific community for many years. Recent work on the various forms of animal and human PKD by different groups with expertise in diverse fields have clearly shown the multifactorial nature of this disease. Strikingly, although caused by different gene defects, the phenotype involves three central elements (**Fig. 1**). First, there is abnormal epithelial cell proliferation of the epithelial lining around the cyst lumen that accounts for the progressive increase in the surface area of the cyst. Second, epithelial cells lining the macroscopic cysts, which are predominant in PKD, show a net trans-epi-

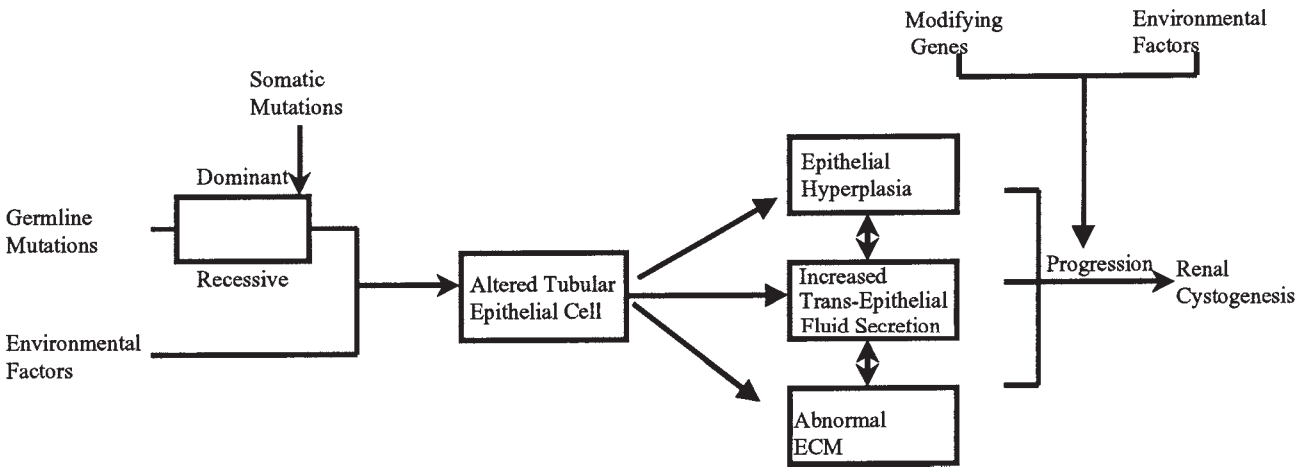

Fig. 1. A schematic representation of the factors that affect the etiology of polycystic kidney disease.

thelial fluid secretion (directed toward the cyst lumen) resulting from a faulty signaling that accounts for the fluid accumulation within the cysts. Third, changes in the tubular basement membrane and the extracellular matrix (ECM) of the expanding cyst could result in the disruption of the cytoskeletal-ECM and the cell-matrix interactions.

As mentioned previously, one of the common features in the various animal models of PKD is the augmented expression of several genes such as c-myc, c-fos, and c-ki-ras which are associated with cellular proliferation. In addition, renal cystic changes occur in transgenic mice that express activated proto-oncogenes (c-myc) and growth factors (hGF), which suggests that cellular proliferation may be the central driving force in cyst formation in PKD.

The factors that convert normally reabsorptive renal epithelial cells into the secretory cells responsible for cyst fluid accumulation have not yet been elucidated. It has been proposed that the epithelial cells of the cyst mimic the behavior of the epithelial cells derived from the intestine, where changes in the state of cellular differentiation may account for the functional differences between secretory and absorptive phenotype. It has therefore been suggested that the Cl^- and fluid secretion by the cystic cells may be the direct result of the inability of tubular epithelial cells to terminally differentiate rather than the presence of abnormal transport mechanism ***(42)***.

ECM composition is known to be important in regulating the growth, shape, and state of differentiation of the overlying epithelial cells. In turn, the state of differentiation of the overlying epithelial cell influences the pattern of ECM synthesis. It has therefore been proposed that defective interactions between the tubular epithelial cells and the ECM may be the initiating event in cyst formation. It is evident that the three central pathogenic characteristics seen in the various animal and human forms of the cystic disease—increased cell proliferation, altered ECM composition, and fluid accumulation—might influence each other and therefore cannot be studied as independent features.

Kidney development begins with the reciprocal interactions between the ureteric bud and the metanephric mesenchyme that lead to condensation of the metanephric mesenchyme, which then aggregates into pretubular clusters and undergoes epithelialization to form renal tubules. Subsequent morphogenesis and differentiation of the tubular epithelium lead to the establishment of a functional nephron ***(43)***. Grantham et al. have suggested that the continued proliferation of the cystic epithelium may be a consequence of the failure of renal tubular epithelial cells to terminally differentiate ***(42)***. This state of immaturity of the renal epithelial cells may either be the result of an arrest in its maturation during renal development or the permanent de-differentiation state of the normal tubular epithelium, acquired as a result of an environmental insult such as an injury. Many of the genes whose mutation lead to PKD in the various animal

models may act in common or interrelated pathways involved in the formation or maintenance of renal tubules.

Future research should be focused on a better understanding of the cascade of pathological events and the normal function of the gene mutated in each of these rodent models of PKD. Deciphering the interrelationship between the various models of PKD will help us deduce biological pathways that are important in maintaining the function/stability of the kidney. Generation and analysis of compound homozygous and heterozygous mutant animals by intercrossing the various mutant models will provide answers regarding the interrelationship between the various models. In addition, modifier genes and environmental factors that are known to alter the severity of the renal disease in some of the animal models can also be used as additional determinants to further define the interrelationship between the various models. These studies will help in the identification of pathways and cellular processes involved in the normal interaction between epithelial cells and their environment and will provide additional avenues to develop therapeutic interventions to treat this devastating human disease.

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