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PROGRESS IN MOLECULAR GENETICS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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1. ABSTRACT

Among the prevalent human genetic disorders, human autosomal dominant polycystic kidney disease is certainly one of the most challenging, both from a clinical and a fundamental perspective. In the recent years, important progress opened novel research avenues to elucidate the genetic basis, the cellular pathophysiologic mechanisms and the molecular function of genes and proteins involved in autosomal dominant polycystic kidney disease.

2. INTRODUCTION

One of the most common genetically transmitted diseases is the human autosomal dominant polycystic kidney disease (ADPKD). ADPKD is fully penetrant but with variable expression, afflicting one in 500 to 1000 individuals. ADPKD is characterized by the presence of many renal epithelial cysts in all parts of the nephron that affect both kidneys. Typically, the cystic dilatations of tubules and of glomeruli initiates *in utero* in ADPKD kidneys (1). Progression of these renal epithelial cysts ultimately modifies and remodels the renal architecture. Consequently, the renal physiologic functions are altered leading to end-stage-renal-failure that accounts for 10% of all individuals requiring renal replacement therapy. This disease has been associated with various extrarenal manifestations such as hepatic cysts and cerebral aneurysm (2). No prevention or cure exists and at the present time, patients receive only supportive/palliative therapy, long term dialysis and transplantation.

Recent advances in the understanding of the genetic basis of human ADPKD have allowed substantial progress and have established the first step in the quest toward a molecular targeted therapy. In parallel, studies and experimentations on animal models can bypass the limitations inherent to human subject and provide insights into the molecular mechanism and development of ADPKD pathophysiology. The aim of this review is to outline our present state of knowledge on ADPKD.

3. CURRENT KNOWLEDGE

3.1. Pathophysiology: cellular and molecular insights

Several pathophysiologic mechanisms have been proposed to explain the origin of renal cyst formation. One of the most frequent and consistent cellular morphologic findings in ADPKD kidneys is increased proliferation of the renal epithelial cells and as such, is considered a potential pathophysiologic mechanism. Indeed, tubular cell proliferation requires accommodation of an increase cell mass that probably modify the renal parenchyma and could lead to cystogenesis. We and others have showed an increased proliferative index in ADPKD kidneys (3, 4). Interestingly, the proliferating cells were frequently found in clusters, suggesting a near neighbor phenomena. The proliferative index was markedly elevated in both non-cystic and cystic epithelia. As elevated proliferation is detected in non-cystic epithelium, this phenomenon precedes cyst formation and may be a primary event for cystogenesis.

The progressive development of renal cyst lesions correlates with a net influx of fluid. Thus an alteration in trans-epithelial tubular transport could also be another pathogenetic mechanism whereby a normal absorptive epithelium would become a cystic secretory epithelium. Similarly, altered epithelial polarity could cause secretion of fluid associated with development and gradual enlargement of renal cysts. In fact, abnormal polarity of Na,K-ATPase (5) and of CFTR (cystic fibrosis transmembrane receptor) (6) was frequently associated with presence of renal cysts but they do not appear to be a prerequisite for cystogenesis or for the subsequent progressive cyst enlargement.

An alteration in tubular basement membrane has been proposed as a possible pathogenetic mechanism (7). Abnormal spatial arrangement and/or protein composition of the tubular and glomerular basement membrane would be likely to compromise the epithelial tubular environment and

could potentially lead to cyst formation. However, these phenotypic modifications are mainly associated with the cystic changes, suggesting that they occur as a secondary phenomenon due to tubular expansion.

We and others have shown evidence for increased apoptosis or programmed cell death in the cystic kidneys from ADPKD patients (4, 8). The apoptotic index was highly elevated in glomeruli, in cystic and non-cystic tubules. Similar to proliferating cells, apoptotic cells were frequently found in clusters, suggesting a cell-cell or cell-matrix interaction. Our molecular studies on ADPKD fetal and adult kidneys showed high expression of the protooncogene c-myc (4). These studies also indicated that the ADPKD apoptotic mechanism is probably independent of the anti-apoptotic factor Bcl-2 and of the pro-apoptotic factor p53. Apoptosis is unlikely to be the sole cellular pathophysiologic mechanism for ADPKD since the kidneys would rather become hypoplasic instead of enlarged. Nevertheless, the occurrence of apoptosis is probably a crucial process in the remodeling of the renal parenchyma. Importantly, kidney tissues with highest number of apoptotic cells also displayed the highest level of proliferation index which suggest that these mechanisms are interrelated.

All of these cellular processes are not mutually exclusive and in fact, may actually participate into a common interplay within renal tubular cyst formation and progression.

3.2. Genetics of human ADPKD

In human, at least two loci have been implicated in the pathogenesis of ADPKD: the PKD1 locus mapped on chromosome 16 (9) and PKD2 on chromosome 4 (10, 11). Evidence for a third locus was provided by the identification of ADPKD patients that are unlinked to both of these loci (12). Mutations in the PKD1 and PKD2 loci, encoding respectively polycystin-1 and polycystin-2 account for ~95% of ADPKD cases. These two genotypes give rise to similar clinical manifestations but in general, individuals affected by PKD1 mutations have a more severe expression of the disease than those with PKD2 mutations. The mean onset of end-stage renal disease in individuals with mutated PKD1 and PKD2 genes is 53 and 69 years respectively (13).

A schematic comparison of our present knowledge on PKD1 and PKD2 genes is summarized in figure 1. The PKD1 gene spans 54kb and contains 46 exons. Approximately two thirds of the PKD1 gene coding sequence located in 16p13.3 is reiterated four times within the proximal 16p13.1 area but contains a unique region at its 3' end. The PKD1 gene encodes a 14kb transcript (14-17). As a consequence of the reiteration, 4 additional transcripts have been identified with 97% of similarity to the fully two thirds of the PKD1 5' end coding sequence. It is unknown yet whether the reiterated gene transcripts are translated into protein. Computer sequence analysis of the polycystin-1, revealed that the amino terminal portion of the protein is made up of a mosaic of previously described domains: leucine-rich repeats flanked by cysteine-rich structures, an LDL-A domain, a C-type lectin domain, suggesting that these extracellular domains may be involved in protein-protein and protein-carbohydrate interactions (figure 1) (16, 17). These domains are followed by 16 PKD repeats, an REJ-like domain (receptor egg jelly), 11 transmembrane domains. The first intracellular/ cytoplasmic loop contains a so-called PLAT domain (for polycystin-1, lipoxygenase and alpha toxin) (18) that is conserved in human pancreatic lipase (19) and rabbit 15-lipoxygenase (20) and alpha toxin from *Clostridium perfringens* (21). This polycystin-1 PLAT domain could be, by analogy to proteins containing these domains, involved in mediating interactions with other membrane proteins. The last intracellular loop contains at the carboxy terminal, a coiled-coil domain comprised of 5 heptad repeats that may form multimeric protein complex and a potential PEST sequence that could target polycystin-1 for degradation. Based on these predictions, the polycystin-1 protein could act as a cell surface receptor or participate in a large membrane complex that could be involved in cell-cell and/or cell-matrix interactions.

The PKD2 gene spans 68kb and contains 15 exons. The PKD2 gene encodes a 5.4kb transcript (22). Polycystin-2 is predicted to contain six putative membrane-spanning domains. While the amino-terminal sequence of the protein has no known homology, the latter part of the protein has significant homology with cation channels such as Ca^{2+} and Na^{+} voltage-activated channels (VAC) and the transient receptor potential channel (TRPC) subunits (figure 1). The carboxy-terminal region of polycystin-2 contains as polycystin-1, a coiled-coil structure that could be implicated in protein interaction *in vivo*.

In vitro studies have been carried out to identify possible interacting proteins with polycystin-1 and polycystin-2 through their coiled-coil structure. These experiments described homodimeric interactions of polycystin-1 and of polycystin-2 (23). In addition, the polycystin-1 carboxy-terminus interacts with that of polycystin-2, suggesting that both could signal through a common pathway (23, 24). Interaction of polycystin-1 with other proteins has also been shown for the G protein, RGS7 (25). The polycystin-2 carboxy terminus has also been shown to associate specifically with the TRPC1 channel (26). Altogether these polycystin proteins may form heteromultimeric complex involved in modulation of calcium entry in response to G-protein coupled receptor.

3.3. Mutations in PKD1 and PKD2 genes

Mutation analysis for the complete PKD1 gene has been hampered by the difficulties in assessing the duplicated region of PKD1. Most of the PKD1 mutations reported to date have been localized to the 3' unique portion of PKD1 (15, 27-30). These mutations include frameshifts, nonsense, missense mutations, splicing mutations, deletions. Recently, mutations in the duplicated region of the PKD1 gene have also been identified (31, 32). PKD1 mutations have been described to produce mainly aberrant transcripts but it is not clear whether these mutations result in the absence of protein or in the production of abnormal protein. Large deletions have also been defined that consists of entire deletion of the PKD1 gene and disrupt the TSC2 gene (tuberous sclerosis) immediately adjacent (53 bp) to the PKD1 gene. This form of contiguous deletion results in a severe PKD phenotype with early onset in childhood (33). At present, most mutations appear unique to an individual family, suggesting a high rate of novel mutations occurring at the PKD1 locus. The occurrence of such an unusual high frequency of mutations would predict particular hot spots within the PKD1 gene which have not yet been defined.

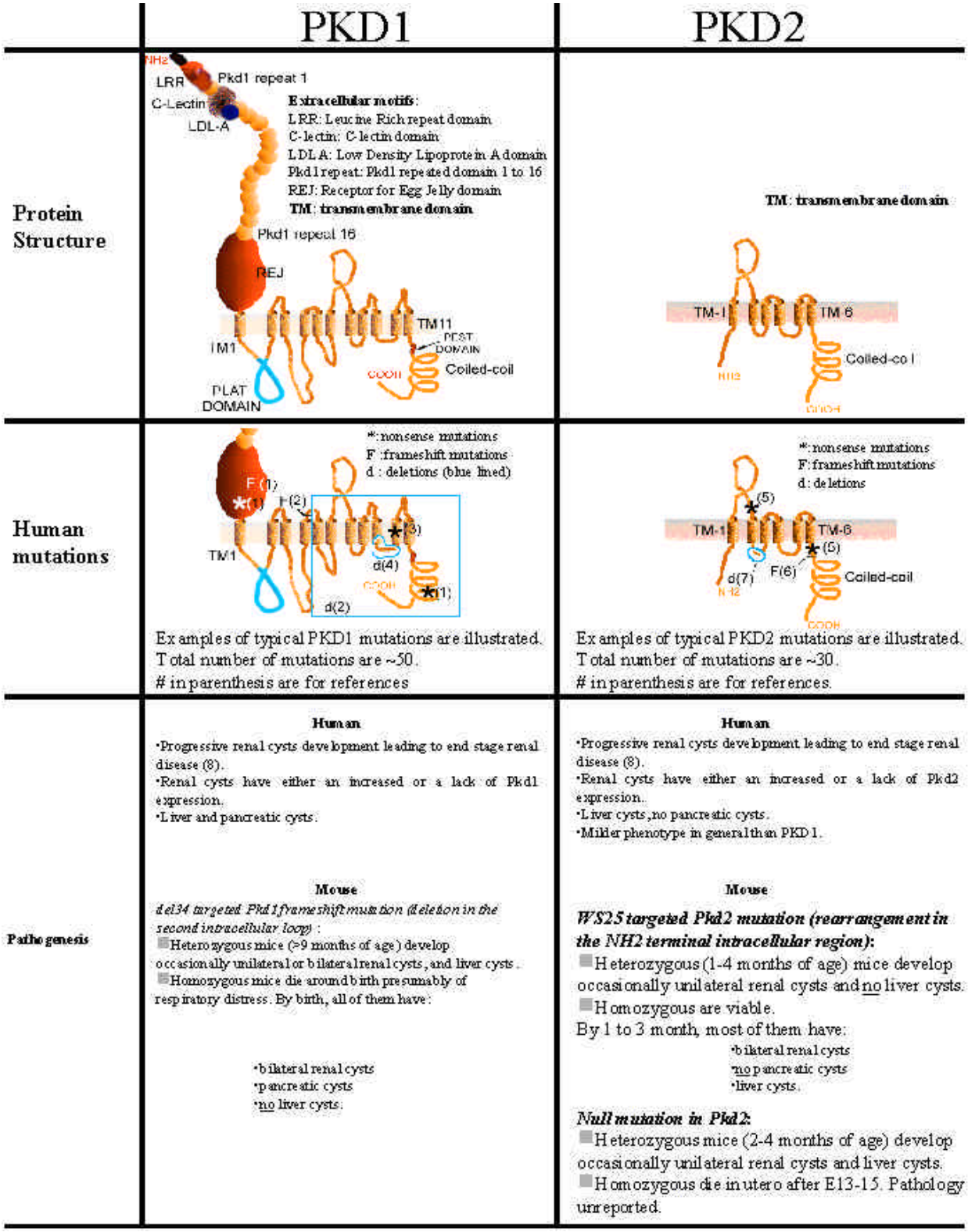

Figure 1. References: (1) Peral *et al.*, 1997. (2) European Polycystic Kidney Disease Consortium, 1994. (3) Turco *et al.*, 1995. (4) Peral *et al.*, 1996. (5) Mochizuki et al., 1996. (6) Pei *et al.*, 1998. (7) Torra *et al.*, 1999. (8) Parfrey *et al.*, 1990.

Mutation screening in the PKD2 locus has shown that a myriad of alterations can occur over the entire gene. Indeed, missense, nonsense, frameshift, deletions, splicing mutations have been described which are likely to produce a truncated product and inactivate the gene (22, 34-38).

In contrast to other genetic diseases, the analysis of PKD1 and PKD2 gene mutations as a tool for prenatal or neonatal diagnosis for ADPKD are technically complicated by the size of the genes and the diversity of mutations. At present, prenatal, neonatal or even adult diagnosis of ADPKD relies on ultrasonography, computed tomography and magnetic resonance imaging of the abdomen to detect renal cysts. The sensitivity of ultrasonography is the preferred method from fetus to adult since it does not require radiation or contrast material, is not invasive and is relatively inexpensive. To increase the probability of accurate diagnosis at young age, the use of genetic linkage analysis to PKD1 and PKD2 genes with DNA probes could complement the phenotypic techniques by identifying individuals at risk of carrying a mutated gene.

3.4. Expression of PKD1 and PKD2 genes and proteins

Human PKD1 expression has been undertaken by analysis of RNA and protein. In the kidneys, we and others have shown moderate RNA expression in normal adult kidneys and increased levels (~2-fold) in ADPKD kidneys (4, 39). Various antibodies have been generated by several research groups to analyze expression of polycystin-1 (39-44). Protein expression was detected during normal renal organogenesis and in fetal glomerular and tubular epithelial cells. In adult, expression in normal kidneys decreased and become more localized to the collecting and distal tubules. In contrast, levels of polycystin-1 appeared to increase in end-stage renal cystic epithelia consistent with RNA expression analysis. In addition to the kidneys, polycystin-1 is also expressed in other epithelia for example the liver and pancreatic ducts, and in non-epithelial tissues such as astrocytes. Reports on the subcellular localization of polycystin-1 are somewhat more heterogenous: some of them show localization to membrane-enriched fractions by Western (40, 42) whereas others show mainly cytoplasmic staining (39, 43). These results can be best explained, if the polycystin-1 protein is continuously recycling within the cell, a process that could be dependent on interaction with cellular and/or extracellular partners.

In human, reports on expression of PKD2 are rather limited. Recently, PKD2 expression have been shown to be similar to PKD1 by immunostaining that is, expressed highly in collecting tubules and moderately in distal tubules of the adult kidneys (45). In contrast to the increased PKD1 expression in renal cysts affected by PKD1 mutations, Pkd2 expression does not appear increased in renal cysts from ADPKD patients caused by PKD2 mutations. In non-renal tissues, PKD2 appears expressed in a wide variety of different cell types.

3.5. Pathogenetic mechanism in ADPKD

As the name implies, ADPKD was initially classified autosomal dominant for genetic transmission of PKD1 and PKD2. At present, the pathogenetic mechanism has been questioned in regards of the mutational process. One hypothetical mechanism, consistent with a dominant mutation, correspond to gene deregulation causing overexpression of the PKD1 gene that could result in a “gain of function” phenotype which is supported by several laboratories (4, 39-42). Although the majority of renal cysts were positive for polycystin-1 expression, it has been reported that a significant minority of cysts was negative (40, 45). Because all cells of the nephrons have inherited the same germ line mutation, it would be expected for a dominant mutation that all nephrons would develop cysts. However, in ADPKD only a proportion of the nephrons acquires tubular cysts, thereby raising questions on the pathogenetic mechanism. Thus, a second hypothetical mechanism of two-hit model was proposed for cystogenesis (46). On this basis, individual ADPKD renal cysts were analyzed and showed loss of heterozygosity (LOH) for the PKD1 wild type gene (47, 48), supporting the two-hit theory. A third possibility involves haploinsufficiency or gene dosage effect that cause a reduction in polycystin-1 production and may lead to imbalance protein levels and alter the formation or role of a polycystin complex. The fourth possibility implicates a mutation in the coding sequences leading to the synthesis of a mutated protein that could interfere with the wild type protein such as a dominant negative effect. Although possible among the known mutations, this last possible mechanism is unlikely to explain the entire spectrum of PKD1 mutations. At present, it is difficult to reconcile all of the known data and to propose a straightforward model of pathogenetic mechanism.

As described for the PKD1 gene, similar observations were made for the PKD2 gene. Somatic inactivation of both PKD2 alleles have been shown in renal cysts from human ADPKD patients (49). However, analysis of ADPKD renal cysts has shown by immunostaining that a majority of cysts expressed polycystin-2 but some cysts lack expression of polycystin-2 (45). These similar findings for PKD2 and PKD1 genes suggest that they may share closely related mechanism of pathogenesis.

3.6. Murine Pkd1 and Pkd2 studies

To gain further insights into PKD1 potential function(s), we and others have analyzed the pattern of expression in murine tissues of Pkd1 by in situ hybridization and immunostaining (50-52). High levels of expression were revealed as early as the murine morula stage. Murine Pkd1 expression in kidneys was determined by immunostaining and in situ hybridization and showed comparable pattern to PKD1 expression in human fetal to adult kidneys. In addition to pattern observed from human tissues, expression of mouse Pkd1 revealed a strong signal in all neural crest cell derivatives and central nervous system. Striking expression was also observed in the aortic arch, developing cartilage, and in condensing mesenchymal cells (50). Quantitative analysis has also revealed Pkd1 expression in the lungs and at a higher level than kidney tissue at all ages. While Pkd1 is widely expressed, it showed a unique tissue specificity and developmental expression pattern. Studies on PKD1/Pkd1 gene expression

and structural homologies suggest that polycystin-1 could be involved in multiple functions.

Because polycystin-1 is expressed in kidney and other branching epithelial tissues, the role of polycystin-1 in branching morphogenesis was investigated on cultured kidney rudiments (53). Since Pkd1 contains 16 PKD repeats, these structural features have been the primary targets for the development of potential competitive inhibitors to alter polycystin-1 function. Soluble peptides derived from a consensus sequence of the PKD repeats were generated and interfered with kidney rudiments development by reducing and modifying the normal renal branching pattern.

In the mouse, Pkd2 protein expression was observed from morula to adulthood (54; Guillaume & Trudel, Mech Dev, in press). In the kidneys, Pkd2 adult expression revealed the most intense signal in the distal tubules, a moderate to low signal in proximal and collecting tubules that slightly differed from the human PKD2 report. In extrarenal tissues, expression was detected in some epithelial and non-epithelial structures such as the pituitary, muscle and red blood cells.

In parallel to murine expression studies, mutation/deletion in the Pkd1 and Pkd2 genes were undertaken to determine polycystin essential role. Targeted disruption was carried out on Pkd1 second intracellular loop by deletion of Pkd1 exon 34 that was predicted to result in a frameshift mutation and absence of full-length polycystin-1. The homozygous mice ($Pkd1^{del34/del34}$) died perinatally with pulmonary hypoplasia, enlarged kidneys and pancreatic cysts (figure 1) (55). These studies provided additional evidence that cystogenesis can arise from a two-hit genetic model mechanism. The development of pulmonary hypoplasia in $Pkd1^{del34/del34}$ mice, when normally high expression of Pkd1 is observed in fetal and newborn lungs associated with the possible role of Pkd1 in branching morphogenesis, suggests that the role of Pkd1 in lung development is crucial for survival. Of interest the $Pkd1^{del34/del34}$ mice showed increased apoptosis, and proliferation as well as elevated c-myc expression, indicating that c-myc can be a molecular target of Pkd1 (56). In addition, the in-depth study of heterozygous mice $Pkd1^{+/del34}$ revealed the presence of renal and liver cysts in 9-14 months old animals (figure 1) (57). In addition to a two-hit theory, these findings can support a pathogenetic model involving a reduction in expression from gene dosage or haploinsufficiency that may lead to cyst formation.

To investigate the role of Pkd2 function, two gene-targeted disruptions were generated in the mouse as summarized in figure 1. The Pkd2 gene was mutated in exon 1 by producing either a somatic unstable allele ($Pkd2^{WS25}$) or a null allele ($Pkd2^{-}$). Heterozygous $Pkd2^{WS25/+}$ mice can develop occasional renal cysts whereas most homozygous $Pkd2^{WS25/WS25}$ mice have bilateral renal cysts and are viable (figure 1) (58). Heterozygous $Pkd2^{-/+}$ mice displayed occasional renal and liver cysts. Interestingly, homozygous $Pkd2^{-/-}$ mice die *in utero* at E13-15 but the cause of death has not been determined and the pathology not reported. Nevertheless, this study demonstrated the essential role of Pkd2 in murine development. Similar to murine Pkd1 gene targeted disruption, Pkd2 studies can support both a second-hit theory for the wild type allele and possibly, a gene dosage or a haploinsufficiency model as pathogenetic mechanisms of cystogenesis.

3.7. Animal models of ADPKD

Studies on human genetic diseases such as sickle cell disease have shown that to progress in understanding the physiopathology of a disease and the development of therapeutic strategies, a suitable animal model is required. Hence, animal models of ADPKD have been generated and intensely studied. Initially, chemically-induced models have been produced in rodents using a variety of agents as glucocorticoids (59, 60), which indicate that environmental factors may play a significant role in the development of renal cysts. Several recessive form, spontaneous or targeted disruption, were characterized by the presence of renal cysts in rodents that more closely resembles the autosomal recessive PKD: the cpk "congenital polycystic kidney" associated with high levels of c-myc expression, $Bcl\text{-}2^{-/-}$, $TG737^{mut}$ (61-69). ADPKD models have also been studied such as the Han:SPRDcy rat model (70, 71). Renal cystic lesions have also been obtained in transgenic mouse approach e.g. simian virus 40 (SV40) or with the HrasT24 oncogene (72-74). Study of these mouse models will provide important insights for the identification of novel proteins involved in the cystogenic pathways and of epistatic modifiers modulating disease severity.

We have generated a transgenic mouse model, SBM, by targeted overexpression of c-myc in renal tubular epithelium which closely resembles human ADPKD (75) All our SBM mice consistently and reproducibly develop severe renal morphological and functional alterations characteristic of PKD and die of renal failure. In SBM mice, tubular and glomerular cysts were detected in utero from E16.5 transgenic fetuses. Extensive glomerular and tubular epithelial hyperplasia and apoptosis were observed in fetal and young transgenic kidneys (76, 77). Adult SBM mice displayed severe glomerulosclerosis and tubular cysts associated with high levels of cell proliferation/hyperplasia and pronounced apoptosis (78). Of importance, the proliferative and apoptotic indexes were greater in young transgenic mice with precystic or early cystic changes than in older mice, indicating that these cellular processes precede cystogenesis. Interestingly, proliferation and apoptosis frequently occurred in cell clusters, suggesting a near neighbor phenomena that recalls human ADPKD cellular patho-morphology. These cellular processes observed in SBM and human ADPKD most likely lead to the continued growth and remodeling of renal cysts. Similarities to human ADPKD also extend to the molecular apoptotic process, as PKD pathogenesis in SBM mice involves a novel c-myc-apoptotic pathway independent of both Bcl-2 and p53. SBM mice also demonstrated altered epithelial polarity for Na,K-ATPase and for various cytoskeletal proteins, fodrin, ankyrin, E-cadherin (79). Typically, SBM macrocysts lost all reactivity to these membrane proteins as well as to tubular segment-specific lectins. This feature is reminiscent of the simultaneous lost of polycystin-1 and polycystin-2 expression observed in human ADPKD renal cysts (45) and suggest that the absence of reactivity may be a feature of advanced renal cysts.

The c-myc deregulation in SBM can confer a dominant form of PKD in mice and clearly demonstrates that c-myc overexpression is a sufficient event to trigger the development of renal cysts. Moreover, the importance of c-myc in PKD development has been demonstrated by several additional findings: 1. increased c-myc expression in the spontaneous cpk mouse with renal cysts (63), 2. increased c-myc expression in the targeted $Pkd1^{del34/del34}$ mice (56), 3. the SBM revertant and their progeny characterized by partial deletion of the transgene, showed no evidence of renal disease (80), 4. mice overexpressing another protooncogene, c-fos in renal epithelium do not develop kidney anomalies (78), 5. human ADPKD renal cysts display increased c-myc expression (4). All of these findings provide compelling evidence for the pertinence of the SBM mouse model.

In SBM and in human ADPKD renal tissues, c-myc expression levels are increased significantly from fetal stage to adulthood, whereas normal c-myc expression is turned off at the onset of terminal differentiation. Expression of c-myc correlates with a concomitant increase in both cell proliferation and apoptosis in cystic renal epithelium, highly reminiscent of the cellular processes occurring during renal organogenesis. Altogether these features support that PKD pathogenesis results from failure to switch out of the renal developmental program.

The similarities between c-myc and polycystin suggest that both proteins are involved in a common signal transduction pathway in both the developing kidney and cystic renal epithelium. Moreover, our present knowledge indicates that c-myc is not only a potential target of Pkd1 as shown by the disruption of the Pkd1 gene in mice but also a critical mediator of cystogenesis as shown by our SBM PKD mouse model.

4. PERSPECTIVES

Understanding the basis of ADPKD is crucial to the development of therapeutic approaches for abrogating or slowing down the progression of the disease. Although the cloning of PKD1 and PKD2 genes has been quite an endeavor, this was a key primary step. Lessons from other human genetic diseases predict that major challenges are still ahead. Many questions remain to be answered not only on the understanding of the direct role of PKD1 and PKD2 in development of ADPKD but also on the molecular pathogenetic mechanism and on the cascade of intracellular and extracellular events implicated in this disease.

Coordinated studies need to be undertaken that include identification of clinical risk factors e.g. markers of disease severity that could serve as predictor in the management of children, identification of ADPKD molecular pathogenesis, characterization of the polycystin structure and cystogenic function. Systematic and thorough studies are essential to pave the way toward development of rational design for adapted treatment and possibly, for gene therapy in future clinical intervention.

5. REFERENCES

1. Michaud, J., Russo, P., Grignon, A., Dallaire, L., Bichet, D., Rosenblatt, D., Lamothe, E. & M. Lambert: Autosomal dominant polycystic kidney disease in the fetus. *Am J Med Genet* 51, 240-246 (1994)

2. Fick, G.M. & P.A. Gabow: Hereditary and acquired cystic disease of the kidney. *Kidney Int* 46, 951-964 (1994)

3. Nadasdy, T., Laszik, Z., Lajoie, G., Blick, K.E., Wheeler, D.E. & F.G. Silva: Proliferative activity of cyst epithelium in human renal cystic diseases. *J Am Soc Nephrol* 5, 1462-1468 (1995)

4. Lanoix, J., D'Agati, V., Szabolcs, M. & M. Trudel: Dysregulation of cellular proliferation and apoptosis mediates human autosomal dominant polycystic kidney disease (ADPKD). *Oncogene* 13, 1153-1160 (1996)

5. Wilson, P.D., Sherwood, A.C., Palla, J.D., Watson, R. & J.T. Norman: Reversed polarity of Na^{+}-K^{-}ATPase: Mislocation to apical plasma membrane. *Am J Physiol* 260, F420-F430 (1991)

6. Brill, S.R., Ross, K.E., Davidow, C.J., Ye, M., Grantham, J.J. & M.J. Caplan: Immunolocalization of ion transport proteins in human autosomal dominant polycystic kidney epithelial cells. *Proc Natl Acad Sci USA* 93, 10206-10211 (1996)

7. Grantham, J.J.: Polycystic kidney disease: Neoplasm in disguise. *Am J Kidney Dis* XV, 110-116 (1990)

8. Woo, D.: Apoptosis and loss of renal tissue in polycystic kidney diseases. *N Eng J Med* 333, 18-25 (1995)

9. Reeders, S.T., Breuning, M.H., Davies, K.E., Nicholls, R.D., Jarman, A.P., Higgs, D.R., Pearson, P.L. & D.J. Weatherall: A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. *Nature* 317, 542-544 (1985)

10. Peters, D.J.M., Spruit, L., Saris, J.J., Ravine, D., Sandkuijl, L.A., Fossdal, R., Boersma, J., Van Eijk, R., Norby, S., Constantinou-Deltas, C.D., Pierides, A., Brissenden, J.E., Frants, R.R., Van Ommen, G.J.B. & M.H. Breuning: Chromosome 4 localization of a second gene for autosomal dominant polycystic kidney disease. *Nat Genet* 5, 359-362 (1993)

11. Kimberling, W.J., Kumar, S., Gabow, P.A., Kenyon, J.B., Connolly, C.J. & S.Somlo: Autosomal dominant polycystic kidney disease: localization of the second gene to chromosome 4q13-q23. *Genomics* 18, 467-472 (1993)

12. Daoust, M., Reynolds, D., Bichet, D.G. & S. Somlo: Evidence for a third Genetic locus for autosomal dominant polycystic kidney disease. *Genomics* 25, 733-736 (1995)

13. Hateboer, N., van Dijk, M.A., Bogdanova, N., Coton, E., Saggar-Malik, A.K., San Millan, J.L., Torra, R., Breuning, M. & D. Ravine: Comparison of phenotypes of polycystic kidney disease types 1 and 2. European PKD1-PKD2 study group. *Lancet* 353, 103-107 (1999)

14. Consortium, E.P.K.D.: Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 75, 1305-1315 (1993)

15. Consortium, E.P.K.D.: The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 77, 881-894 (1994)

16. Burn, T.C., Connors, T.D., Dackowski, W.R., Petry, L.R., Van Raay, T.J., Millholland, J.M., Venet, M., Miller, G., Hakim, R.M., Landes, G.M., Klinger, K.W., Qian, F., Onuchic, L.F., Watnick, T., Germino, G.G. & N.A. Doggett: Analysis of the genomic sequence for the autosomal dominant polycystic kidney disease (PKD1) gene predicts the presence of a leucine-rich repeat. *Hum Mol Genet* 4, 575-582 (1995)

17. Consortium, I.P.K.D.: Polycystic Kidney Disease: The complete structure of the PKD1 gene and its protein. *Cell* 81, 289-298 (1995)

18. Bateman, A. & R. Sandford: The PLAT domain: a new piece in the PKD1 puzzle. *Curr Biol* 9, R588-R590 (1999)

19. van Tilbeurgh, H., Egloff, M.P., Martinez, C., Rugani, N., Verger, R. & C. Cambillau: Interfacial activation of the lipase-procolipase complex by mixed micelles revealed by X-ray crystallography. *Nature* 362, 814-820 (1993)

20. Gillmor, S.A., Villasenor, A., Fletterick, R., Sigal, E. & M.F. Browner: The structure of mammalian 15-lipoxygenase reveals similarity to the lipases and the determinants of substrate specificity. *Nat Struct Biol* 4, 1003-1009 (1998)

21. Naylor, C.E., Eaton, J.T., Howells, A., Justin, N., Moss, D.S., Titball, R.W. & A.K. Basak: Structure of the key toxin in gas gangrene. *Nat Struct Biol* 5, 738-746 (1998)

22. Mochizuki, T., Wu, G., Hayashi, T., Xenophontos, S., 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. & S. Somlo: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272, 1339-1342 (1996)

23. Qian, F., Germino, F.J., Cai, Y., Zhang, X., Somlo, S. & G.G. Germino: PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat Genet* 16, 179-183 (1997)

24. Tsiokas, L., Kim, E., Arnould, T., Sukhatme, V.P. & G. Walz: Homo- and heterodimeric interactions between the gene product of PKD1 and PKD2. *Proc Natl Acad Sci USA* 94, 6965-6970 (1997)

25. Kim, E., Arnould, T., Sellin, L., Benzin, g.T., Comella, N., Kocher, O., Tsiokas, L., Sukhatme, V.P. & G. Walz: Interaction between RGS7 and polycystin. *Proc Natl Acad Sci USA* 96, 6371-6376 (1999)

26. Tsiokas, L., Arnould, T., Zhu, C., Kim, E., Walz, G. & V.P. Sukhatme: Specific association of the gene product of PKD2 with the TRPPC1 channel. *Proc Natl Acad Sci USA* 96, 3934-3939 (1999)

27. Peral, B., Gamble, V., San Millan, J.L., Strong, C., Sloane-Stanley, J., Moreno, F. & P.C. Harris: Splicing mutations of the polycystic kidney disease 1 (PKD1) gene induced by intronic deletion. *Hum Mol Genet* 4, 569-574 (1995)

28. Turco, A.E., Rossetti, S., Bresin, E., Corra, S., Gammaro, L., Maschio, G. & P.F. Pignatti: A novel nonsense mutation in the PKD1 gene (C3817T) is associated with autosomal dominant polycystic kidney disease (ADPKD) in a large three-generation Italian family. *Hum Mol Genet* 4, 1331-1335 (1995)

29. Peral, B., Ong, A.C.M., San Millan, J.L., Gamble, V., Rees, L. & P.C. Harris: A stable, nonsense mutation associated with a case of infantile onset polycystic kidney disease 1 (PKD1). *Hum Mol Genet* 5, 539-542 (1996)

30. Peral, B., San Millan, J.L., Ong, A.C.M., Gamble, V., Ward, C.J., Strong, C. & P.C. Harris: Screening the 3' region of the Polycystic Kidney Disease 1 (PKD1) gene reveals six novel mutations. *Am J Hum Genet* 58, 86-96 (1996)

31. Koptides, M., Constantinides, R., Kyriakides, G., Hadjigavriel, M., Patsalis, P.C., Pierides, A. & C.C. Deltas: Loss of heterozygosity in polycystic kidney disease with a missense mutation in the repeated region of PKD1. *Hum Genet* 103, 709-717 (1998)

32. Thomas, R., McConnell, R., Whittacker, J., Kirkpatrick, P., Bradley, J. &, R. Sandford: Identification of mutations in the repeated part of the autosomal dominant polycystic kidney disease type 1 gene, PKD1, by long-range PCR. *Am J Hum Genet* 65, 39-49 (1999)

33. Brook-Carter, P.T., Peral, B., Ward, C.J., Thompson, P., Hughes, J., Maheshwar, M.M., Nellist, M., Gamble, V., Harris, P.C. & J.R. Sampson: Deletion of the TSC2 and PKD1 genes associated with severe infantile polycystic kidney disease - a contiguous gene syndrome. *Nat Genet* 8, 328-332 (1994)

34. Pei, Y., He, N., Kasenda, M., Wang, K.R., Paterson, A., Liang, Y., Chan, G., Roscoe, J., Brissenden, J., Hefferton, D., Parfrey, P., Somlo, S. & P. St. George-Hyslop: A spectrum of mutations in the polycystic kidney disease 2 (PKD2) gene from eight Canadian kindreds. *J Am Soc Nephrol* 9, 1853-1860 (1998)

35. Pei, Y., Wang, K., Kasenda, M., Paterson, A.D., Liang, Y., Huang, E., Lian, J., Rogovea, E., Somlo, S. & P. St. George-Hyslop: A novel frameshift mutation induced by an adenosine insertion in the polycystic kidney disease 2 (PKD2) gene. *Kidney Int* 53, 1127-1132 (1998)

36. Veldhuisen, B., Saris, J.J., de Haij, S., Hayashi, T., Reynolds, D.M., Mochizuki, T., Elles, R., Fossdal, R., Bogdanova, N., van Dijk, M.A., Coto, E., Ravine, D., Norby, S., Verellen-Dumoulin, C., Breuning, M.H., Somlo, S. & D.J.M. Peters: A spectrum of mutations in the second gene for autosomal dominant polycystic kidney disease (PKD2). *Am J Hum Genet* 61, 547-555 (1997)

37. Viribay, M., Hayashi, T., Telleria, D., Mochizuki, T., Reynolds, D.M., Alonso, R., Lens, X.M., Moreno, F., Harris, P.C., Somlo, S. & J.L. San Millan: Novel stop and frameshifting mutations in the autosomal dominant polycystic

kidney disease 2 (PKD2) gene. *Hum Genet* 101, 229-234 (1997)

38. Xenophontos, S., Constantinides, R., Hayashi, T., Mochizuki, T., Somlo, S., Pierides, A. & C.C. Deltas: A translation frameshift mutation induced by a cytosine insertion in the Polycystic Kidney Disease 2 gene (PKD2). *Hum Mol Genet* 6, 949-952 (1997)

39. Ward, C.J., Turley, H., Ong, A.C.M., Comley, M., Biddolph, S., Chetty, R., Ratcliffe, P.J., Gatter, K. & P.C. Harris: Polycystin, the polycystic kidney disease 1 protein, is expressed by epithelial cells in fetal, adult and polycystic kidney. *Proc Natl Acad Sci USA* 93, 1524-1528 (1996)

40. Geng, L., Segal, Y., Peissel, B., Deng, N., Pei, Y., Carone, F., Rennke, H.G., Glücksmann-Kuis, A.M., Schneider, M.C., Ericsson, M., Reeders, S.T. & J. Zhou: Identification and localization of polycystin, the PKD1 gene product. *J Clin Invest* 98, 2674-2682 (1996)

41. Ibraghimov-Beskrovnaya, O., Dackowski, W.R., Foggensteiner, L., Coleman, N., Thiru, S., Petry, L.R., Burn, T.C., Connors, T.D., Van Raay, T., Bradley, J., Qian, F., Onuchic, L.F., Watnick, T.J., Piontek, K., Hakim, R.M., Landes, G.M., Germino, G.G., Sandford, R. & K.W. Klinger: Polycystin: *In vitro* synthesis, *in vivo* tissue expression, and subcellular localization identifies a large membrane-associated protein. *Proc Natl Acad Sci USA* 94, 6397-6402 (1997)

42. Van Adelsberg, J., Chamberlain, S. & V. D'Agati: Polycystin expression is temporally and spatially regulated during renal development. *Am J Physiol* 272, F602-F609 (1997)

43. Peters, D.J.M., Spruit, L., Klingel, R., Prins, F., Baelde, H.J.J., Giordano, P.C., Bernini, L.F., de Heer, E., Breuning, M.H. & J.A. Bruijn: Adult, fetal, and polycystic kidney expression of polycystin, the polycystic kidney disease-1 gene product. *Lab Invest* 75, 221-230 (1996)

44. Paulson, R., Sharma, C.P., Kim, K., McLaughlin, M., Brown, D. & M.A. Arnaout: Characterization and cell distribution of polycystin, the product of autosomal dominant polycystic kidney disease gene 1. *Mol Med* 2, 702-711 (1996)

45. Ong, A.C.M., Ward, C.J., Butler, R.J., Biddolph, S., Bowker, C., Torra, R., Pei, Y. & P.C. Harris: Coordinate expression of the autosomal dominant polycystic kidney disease proteins, polycystin-2 and polycystin-1, in normal and cystic tissue. *Am J Pathol* 154, 1721-1729 (1999)

46. Reeders, S.T.: Multilocus polycystic disease. *Nat Genet* 1, 235-237 (1992)

47. Qian, F., Watnick, T.J., Onuchic, L.F. & G.G. Germino: The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* 87, 979-987 (1996)

48. Brasier, J.L. & E.P. Henske: 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 (1997)

49. Koptides, M., Hadjimichael, C., Koupepidou, P., Pierides, A. & C. Deltas: Germinal and somatic mutations in the *PKD2* gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum Mol Genet* 8, 509-513 (1999)

50. Guillaume, R., D'Agati, V., Daoust, M. & M. Trudel: Murine Pkd1 is a developmentally regulated gene from morula to adulthood: Role in tissue condensation and patterning. *Dev Dyn* 214, 337-348 (1999)

51. Geng, L., Segal, Y., Pavlova, A., Barros, E.J.G., Löhning, C., Lu, W., Nigam, S.K., Frischauf, A.-M., Reeders, S.T. & J. Zhou: Distribution and developmentally regulated expression of murine polycystin. *Am J Physiol* 272, F451-F459 (1997)

52. Griffin, M.D., O'Sullivan, D.A., Torres, V.E., Grande, J.P., Kanwar, Y.S. & R. Kumar: Expression of polycystin in mouse metanephros and extra-metanephric tissues. *Kidney Int* 52, 1196-1205 (1997)

53. Van Adelsberg, J.: Peptides from the PKD repeats of polycystin, the PKD1 gene product, modulate pattern formation in the developing kidney. *Dev Gen* 24, 299-308 (1999)

54. Markowitz, G.S., Cai, Y., Li, L., Wu, G., Ward, L., Somlo, S. & V. D'Agati: Polycystic-2 expression is developmentally regulated. *Am J Physiol (Renal Physiol)* 46, F17-F25 (1999)

55. Lu, W., Peissel, B., Babkhanlou, H., Pavlova, A., Geng, L., Fan, X., Larson, C., Brent, G. & J. Zhou: Perinatal lethality with kidney and pancreas defects in mice with a targetted PKD1 mutation. *Nat Genet* 17, 179-181 (1997)

56. Fan, X., Lu, W., Reeders, S.T. & J. Zhou.: Enhanced apoptosis in *Pkd1* targeted mice. *J Am Soc Nephrol* 9, #1900 (abstract) (1998)

57. Lu, W., Fan, X., Basora, N., Babakhanlou, H., Law, T., Rifai, N., Harris, P.C., Perez-Atayde, A.R., Rennke, H.G. & J. Zhou: Late onset of renal and hepatic cysts in *Pkd1*-targeted heterozygotes. *Nat Genet* 21, 160-161 (1999)

58. Wu, Q., D'Agati, V., Cai, Y., Markowitz, G., Park, J.H., Reynolds, D.M., Maeda, Y., Le, T.C., Hou JR., H., Kucherlapati, R., Edelmann, W. & S. Somlo: Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* 93, 177-188 (1998)

59. Baylis, C. & B.M. Brenner: Mechanism of the glucocorticoid-induced increase in glomerular filtration rate. *Am J Physiol* 234, F166-F170 (1978)

60. Perey, D.Y.E., Herdman, R.C. & R.A. Good: Polycystic renal disease: A new experimental model. *Science* 158, 494-496 (1967)

61. Preminger, G.M., Koch, W.E., Fried, F.A., McFarland, E., Murphy, E.D. & J. Mandell: Murine congenital polycystic kidney disease: A model for studying development of cystic disease. *J Urol* 127, 556-560 (1982)

62. Nidess, R., Koch, W.E., Fried, F.A., McFarland, E. & J. Mandell: Development of the embryonic murine kidney in normal and congenital polycystic kidney disease: Characterization of a proximal tubular degenerative process as the first observable light microscopic defect. *J Urol* 131, 156-162 (1984)

63. Cowley, B.D., Smardo, F.L., Grantham, J.J. & J.P. Calvet: Elevated c-myc protooncogene expression in autosomal recessive polycystic kidney disease. *Proc Natl Acad Sci USA* 84, 8394-8398 (1987)

64. Moyer, J.H., Lee-Tischler, M.J., Kwon, H.Y., Schrick, J.J., Avner, E.D., Sweeney, W.E., Godfrey, V.L., Cacheiro, N.L.A., Wilkinson, J.E. & R.P. Woychik: Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. *Science* 264, 1329-1333 (1994)

65. Veis, D.J., Sorenson, C.M., Shutter, J.R. & S.J. Korsmeyer: Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75, 229-240 (1993)

66. Nakayama, K., Nakayama, K.I., Negishi, I., Kuida, K., Sawa, H. & D.Y. Loh: Targeted disruption of bcl-2ab in mice: occurrence of gray hair, polycystic kidney disease, and lymphocytopenia. *Proc Natl Acad Sci USA* 91, 3700-3704 (1994)

67. Kamada, S., Shimono, A., Shinto, Y., Tsujimura, T., Takahashi, T., Noda, T., Kitamura, Y., Kondoh, H. & Y. Tsujimoto: bcl-2 deficiency in mice leads to pleiotropic abnormalities: accelerated lymphoid cell death in thymus and spleen, polycystic kidney, hair hypopigmentation and distorted small intestine. *Cancer Res* 55, 354-359 (1995).

68. Sorenson, C.M., Rogers, S.A., Korsmeyer , S.J. & M.R. Hammerman: Fulminant metanephric apoptosis and abnormal kidney development in bcl-2-deficient mice. *Am J Physiol* 37, F73-F81 (1995)

69. Janaswami, P.M., Birkenmeier, E.H., Cook, S.A., Rowe, L.B., Bronson, R.T. & M.T. Davisson: Identification and genetic mapping of a new polycystic kidney disease on mouse chromosome 8. *Genomics* 40, 101-107 (1997)

70. Takahashi, H., Ueyama, Y., Hibino, T., Kuwahara, Y., Suzuki, S., Hioki, K. & N. Tamaoki: A new mouse model of genetically transmitted polycystic kidney disease. *J Urol* 135, 1280-1283 (1986)

71. Ogborn, M.R., Sareen, S. & B. Pinette: Cilazapril delay progression of hypertension and uremia in rat polycystic kidney disease. *Am J Kidney Dis* 26, 942-946 (1995)

72. Brinster, R.L., Chen, H.Y., Messing, A., Van Dyke, T., Levine, A. & R.D. Palmiter: Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. *Cell* 37, 367-379 (1984)

73. McKay, K., Striker, L.J., Pinkert, C.A., Brinster, R.L. & G.E. Striker: Glomerulosclerosis and renal cysts in mice transgenic for the early region of SV40. *Kidney Int* 32, 827-837 (1987)

74. Schaffner, D.L., Barrios, R., Massey, C., Banez, E.I., Ou, C.-N., Rajagopalan, S., Aguilar-Cordova, E., Lebovitz, R.M., Overbeek, P.A. & M.W. Lieberman: Targeting of the rasT24 oncogene to the proximal convoluted tubules in transgenic mice results in hyperplasia and polycystic kidneys. *Am J Pathol* 142, 1051-1060 (1993)

75. Trudel, M., D'Agati, V. & F. Costantini: C-myc as an inducer of polycystic kidney disease in transgenic mice. *Kidney Int* 39, 665-671 (1991)

76. Trudel, M. & V. D'Agati: A model of polycystic kidney disease in SBM transgenic mice. In: Contribution to nephrology : Polycystic kidney disease. Ed: Berlyne, G.M. Contrib. Nephrol., Karger, S., Basel, Brooklyn, NY, vol. 97, 7-59 (1992)

77. Trudel, M., Barisoni, L., Lanoix, J. & V. D'Agati: Polycystic Kidney Disease in SBM Transgenic Mice: Role of c-myc in Disease Induction and Progression. *Am J Pathol* 152, 219-229 (1998)

78. Trudel, M., Lanoix, J., Barisoni, J., Blouin, M.-J., Desforges, M., L'Italien, C. & V. D'Agati: C-MYC-induced Apoptosis in Polycystic Kidney Disease is Bcl-2 and p53 Independent. *J Exp Med* 186, 1873-1884 (1997)

79. Barisoni, L., Trudel, M., Chretien, N., Ward, L., Adelsberg, J.V. & V. D'Agati: Analysis of the role of membrane polarity in polycystic kidney disease of transgenic SBM mice. *Am J Pathol* 147, 1728-1735 (1995)

80. Trudel, M., Chretien, N. & V. D'Agati: Disappearance of polycystic kidney disease in revertant c-myc transgenic mice. *Mamm Genome* 5, 149-152 (1994)

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