Tubular Obstruction Leads to Progressive Proximal Tubular Injury and Atubular Glomeruli in Polycystic Kidney Disease

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In polycystic kidney disease (PKD), renal parenchyma is destroyed by cysts, hypothesized to obstruct nephrons. A signature of unilateral ureteral obstruction, proximal tubular atrophy leads to formation of atubular glomeruli. To determine whether this process occurs in PKD, kidneys from pcy mice (moderately progressive PKD), kidneys from cpk mice (rapidly progressive PKD), and human autosomal dominant PKD were examined in early and late stages. Integrity of the glomerulotubular junction and proximal tubular mass were determined in sections stained with Lotus tetragonolobus lectin. Development of proximal tubular atrophy and atubular glomeruli was determined in serial sections of individual glomeruli. In pcy mice, most glomerulotubular junctions were normal at 20 weeks, but by 30 weeks, 56% were atrophic and 25% of glomeruli were atubular; glomerulotubular junction integrity decreased with increasing cyst area ($r = 0.83, P < 0.05$). In cpk mice, all glomerulotubular junctions were normal at 10 days, but by 19 days, 26% had become abnormal. In early-stage autosomal dominant PKD kidneys, 50% of glomeruli were atubular or attached to atrophic tubules; in advanced disease, 100% were abnormal. Thus, proximal tubular injury in cystic kidneys closely parallels that observed with ureteral obstruction. These findings support the hypothesis that, in renal cystic disorders, cyst-dependent obstruction of medullary and cortical tubules initiates a process culminating in widespread destruction of proximal convoluted tubules at the glomerulotubular junction.

Support: NIH grants P50 DK096373 (R.L.C.) and RO2DK081579 (D.P.W.) and a University of Virginia Children’s Hospital grant (R.L.C.). Portions of this study were presented at the American Society of Nephrology, San Diego, CA, November 2012, the Pediatric Academic Societies meeting, Washington, DC, May 2013, and the International Workshop on Developmental Nephrology, Edinburgh, Scotland, June 2013. Disclosures: None declared.
kidney disease. The renal response to UO has uncovered a rich array of anatomical and biochemical changes that result from stopping the flow of urine by surgical ureteral obstruction. These cellular responses likely represent ineffective reparative responses to urinary obstruction. Morphometric studies of renal injury in the UO model reveal that oxidative stress and cell death and remodeling at the glomerulotubular junction (GTJ) lead to the complete separation of the glomerulus from the proximal tubule (atubular glomeruli). Although similarities in the biochemical features of UUO and PKD were pointed out in 2000, it was not until the disappearance of proximal tubule mass secondary to cell death was clearly defined that the potential connection was appreciated more widely by those working in the PKD field. The current study applies the methods developed in the murine UUO model to renal cystic disorders to determine whether progressive tubule obstruction could contribute to the loss of renal function.

Atubular glomeruli have been reported in the Cy rat, a model of ADPKD in which cysts develop in proximal tubules; however, atubular glomeruli have not been described in human ADPKD or other genetic causes of PKD. To determine whether GTJ atrophy and the formation of atubular glomeruli are a common feature of cystic kidneys and follow a course similar to UUO, two murine PKD models were investigated: the pcy mouse, which develops moderately progressive, late-onset disease (analogous to human ADPKD); and the cpk mouse, which develops rapidly progressive, early-onset disease (analogous to human ARPKD). In addition, relatively early- and late-stage human ADPKD kidneys were examined for evidence of atrophy in the proximal convoluted tubules, GTJ abnormalities, and the presence of atubular glomeruli.

Materials and Methods

Mouse

All mice were bred and sacrificed for study in accordance with an animal protocol approved by the Kansas University Medical Center (Kansas City) Institutional Animal Care and Use Committee, in compliance with the NIH Guide for the Care and Use of Laboratory Animals. Kidneys from cpk mice were studied at 10 and 19 days of age (early maturation; n = 1 at each age); for serial section study (see details later), 21 glomeruli were examined at 10 days, and 27 glomeruli were examined at 19 days. Kidneys from pcy mice were studied at 1, 4, 5, 9, 10, 20, and 30 weeks of age (n = 1 to 3 mice at each age). For serial section study, at 4 weeks, 23 glomeruli were examined; at 9 weeks, 73 glomeruli were examined; and at 30 weeks, 16 glomeruli were examined. C57BL/6 mice were used as controls for cpk mice, and CD-1 mice were used as controls for the pcy mice.

Human Samples

Nephrectomy specimens were obtained from the PKD Research Biomaterials and Cellular Models Core at the Kansas University Medical Center Kidney Institute. Kidneys were surgically removed from ADPKD patients with relatively early-stage disease [1 = 34-year-old female; serum creatinine, 1.3 mg/dL (ruptured cerebral aneurysm); 2 = 41-year-old female; serum creatinine, 1.9 mg/dL (unmanageable pain)]; and late-stage disease (3 = 40-year-old male dialysis patient). The protocol for the use of Surgically discarded human kidney tissues complies with the Declaration of Helsinki and with federal regulations, and was approved by the Institutional Review Board at Kansas University Medical Center.

Kidney Tissue Processing

Formalin-fixed tissues, embedded in paraﬁn, were divided into sections (4 μm thick for mouse kidney or 5 μm thick for human kidney). Formation of atubular glomeruli was examined by serial section analysis in kidneys from mice with early (cpk) or late (pcy) development of cysts, and in nephrectomy tissue from the three adult ADPKD patients.

Morphometric Analysis of Proximal Tubules and Cyst Size

Mouse kidney sections were stained with Lotus tetragonolobus agglutinin to identify normal proximal tubule epithelial cells and mature columnar cells of Bowman’s capsule, indicative of intact GTJ. Lotus stains mature proximal tubules and papillary collecting ducts, but not loops of Henle or the principal cells of cortical and outer medullary collecting ducts. Therefore, Lotus staining is useful for quantifying the extent of parenchymal preservation in the renal cortex. Mice of the following ages were examined: cpk, 10 days (n = 1) and 19 days (n = 1); pcy, 1 week (n = 2), 4 weeks (n = 3), 5 weeks (n = 1), 9 weeks (n = 1), 10 weeks (n = 3), 20 weeks (n = 3), and 30 weeks (n = 3). Two sections of each kidney were examined for each parameter (the volume fraction of proximal tubules [VvPT]), Lotus positivity percentage in glomerular capsules, and cyst area percentage), and the results were averaged for that animal. All glomeruli in a single midcoronal section of each kidney were counted and scored on the basis of percentage of Lotus positivity in glomerular capsules. Additional measurements were made with ImagePro Plus version 5.1 image-analysis software (Media Cybernetics, Silver Spring, MD). For these, three identical micrographs were taken of each of 10 random fields per kidney. One micrograph was used to measure VvPT, as described previously, the second for tracing and measuring the area occupied by cysts, and the third for determining the total area of each field under examination. The tissue-specific proximal tubule contribution was calculated by dividing VvPT/the total area minus the cyst area.

The contribution of cysts was determined by measuring all cyst profiles in a single section of each kidney at ×40 magnification; depending on the size of the kidneys, one to
four nonoverlapping micrographs were necessary to capture the entire section. The individual cyst areas were measured with ImagePro software, version 5.1 (Media Cybernetics) and totaled, then divided by the total kidney section area so as to be expressed as percentages of total kidney area.

Serial Section Analysis of GTJs

Multiple glomeruli were examined in a single mouse (or patient) from representative stages of progression. Because this required the analysis of >8000 glomerular profiles, additional individuals were not included for this portion of the study. As reported previously in mice subjected to UUO,7 and discussed later, there is a close correlation between the fraction of glomeruli with normal GTJ and Lotus lectin staining. Serial sections were examined, as described previously,7 in 21 glomeruli from a 10-day-old and 27 glomeruli from a 19-day-old cpk mouse, as well as 23 glomeruli from a 4-week-old, 73 glomeruli from a 9-week-old, and 16 glomeruli from a 30-week-old pcy mouse, compared with 15 glomeruli from a CD-1 mouse. In mice, glomerular morphological characteristics were examined in approximately 30 consecutive coronal sections of each glomerulus at a total magnification of ×400 and scored as normal, atrophic (connected to thinned or collapsed proximal tubules), or atubular (lacking any connection to proximal tubules) (Figures 1, 2, and 3). PAS staining of sections was used to intensify basement membrane material to aid both in detecting tubule basement membrane thickening (indicative of atrophy) and in the tracing of GTJs. Human nephrectomy specimens were categorized as either pre-end stage ADPKD (two specimens) or end-stage ADPKD (one specimen); 20 glomeruli were analyzed in each specimen. Approximately 60 serial sections were required to encompass each human glomerulus. Human glomeruli generally lack columnar parietal epithelial cells lining the urinary pole; the presence of tubule atrophy was recorded in PAS-stained kidneys. Atubular glomeruli were recorded in relation to the total number of glomeruli examined.

Figure 1 Changes in kidney morphological characteristics with age in pcy mice: L. tetragonolobus staining of kidney sections of pcy mice at 5 (A) and 30 (B) weeks of age compared with an example of a normal parent strain (CD-1; C). D and E: Details of 5-week-old pcy kidney (Lotus staining). D: Several cystic profiles are evident in the papilla (papillary collecting ducts are also stained by Lotus). E: Cortical parenchyma retains limited numbers of proximal tubules and glomeruli (containing Lotus-positive epithelial cells in its capsule.). F and G: Details of 30-week-old pcy kidney. F: A large cyst has deformed adjacent collecting-duct profiles in the papilla. Note atrophic tubules above and adjacent to the cyst. G: A remaining region of cortical parenchyma with intact proximal tubules and glomeruli. Most cysts in this field contain Lotus-positive cells in their walls, identifying them as intercalated cells, and the cysts as being derived from cortical collecting ducts. H–K: Representative profiles of glomeruli in L. tetragonolobus–stained tissue sections that illustrate structural criteria, which define categories of glomerulotubular degeneration. H and I: Arrows point to cells in glomerular capsules that react with Lotus lectin; thus, both these glomeruli would be scored as Lotus positive. J and K: Glomeruli are Lotus negative. Although the glomerulus in J is surrounded by Lotus-positive proximal tubule profiles, the atubular glomerulus in K is not (confirmed in serial sections as atubular). Scale bars: 500 μm (D and F); 100 μm (E and G); 50 μm (K, applying to H–K). G, glomeruli.
Statistical Analysis

Polynomial regression analysis was used to characterize the change in fractional total cyst area in relation to age (Figure 2A). Linear regression analysis was performed to describe the change in fraction of *Lotus*-positive glomeruli in relation to the fractional total cyst area (Figure 2D).

Results

Proximal Tubular Injury Progresses Gradually in the pcy Mouse

In the *pcy* mouse, renal cysts progressively develop, starting in the neonatal period and continuing through adulthood. At 5 weeks of age, cysts varied greatly in size and were distributed throughout the parenchyma (Figure 1A), and cystic expansion appeared to increase by 30 weeks of age (Figure 1B). Staining with *L. tetragonolobus* lectin identifies collecting ducts in the papilla (Figure 1, D and F) and mature proximal tubules in the cortex (Figure 1, E and G). Compression of residual *Lotus*-positive papillary collecting ducts was observed at 30 weeks (Figure 1F). However, there was preservation of large areas of normal-appearing parenchyma at 5 weeks (Figure 1E) through 20 weeks of age (data not shown), with smaller areas of parenchyma interspersed, with cysts remaining after 30 weeks (Figure 1G).

A variety of glomerulotubular profiles could be observed (Figure 1, H–K). *Lotus* lectin bound to mature proximal tubular epithelial cells, which were contiguous with tall parietal epithelial cells normally extending around the urinary pole of Bowman’s capsule in the mouse (Figure 1H). With progression of disease, tubular atrophy at the GTJ resulted in gradual disappearance of *Lotus* staining (Figure 1, I and J), and disconnection of the glomerulus from the proximal tubule (Figure 1K).

Quantification of the histomorphometric changes in the renal parenchyma of *pcy* mice demonstrated that there was a marked increase in total cyst area between 20 and 30 weeks (Figure 2A). However, there was preservation of large areas of normal-appearing parenchyma at 5 weeks (Figure 1E) through 20 weeks of age (data not shown), with smaller areas of parenchyma interspersed, with cysts remaining after 30 weeks (Figure 1G). A variety of glomerulotubular profiles could be observed (Figure 1, H–K). *Lotus* lectin bound to mature proximal tubular epithelial cells, which were contiguous with tall parietal epithelial cells normally extending around the urinary pole of Bowman’s capsule in the mouse (Figure 1H). With progression of disease, tubular atrophy at the GTJ resulted in gradual disappearance of *Lotus* staining (Figure 1, I and J), and disconnection of the glomerulus from the proximal tubule (Figure 1K).
through 30 weeks (Figure 2B). Proximal tubular volume fraction in pcy mutants was moderately reduced in the first 10 weeks and then decreased markedly by 30 weeks (Figure 2B). The fraction of pcy mouse glomeruli with mature intact GTJ paralleled that of wild-type animals through 10 weeks of age, but then decreased precipitously (Figure 2C). There was a significant negative correlation between the fraction of Lotus-positive glomeruli and total cyst area ($r = 0.83$, $P < 0.05$) (Figure 2D).

Analysis of serial sections through glomeruli from pcy mice at 4, 9, and 30 weeks of age revealed a progression of injury to the glomerulotubular junction, leading to the formation of atubular glomeruli (Figure 2E). At 4 weeks of age, 86% of 22 glomeruli had intact GTJ, with 10% being atrophic and 4% being atubular, whereas at 9 weeks, 74% of 73 glomeruli had intact GTJ, 8% had atrophic GTJ, and 18% had atubular GTJ. By contrast, after 30 weeks, pcy mice had only 19% of 16 glomeruli with normal GTJ, with most (56%) connected to atrophic proximal tubules, and 25% were atubular. In kidneys of wild-type CD-1 mice, 100% of the 15 glomeruli were normal at 30 weeks of age. Figure 3 provides an illustration of the glomerulotubular scoring using PAS staining (Figure 3A) and the serial section analysis of an atubular glomerulus (Figure 3B) in a 9-week-old pcy mouse.

Rapid Cyst Formation and Death Precede Full Evolution of Proximal Tubular Injury in the cpk Mouse

Multiple cysts were scattered throughout the cpk mouse kidney at 10 days of age (Figure 4A); the largest cysts were
found in the medulla. At 10 days, most *Lotus* lectin-positive proximal tubules in the cpk kidney appeared normal, but some were dilated (Figure 4D). By 19 days, cysts had enlarged dramatically and were evenly distributed throughout the cortex and medulla (Figure 4, B and E). No cysts were observed in kidneys from wild-type mice (Figure 4C). As shown in a previous report, there is significant maturation of *Lotus*-positive proximal tubules during normal development from 10 to 19 days, as also observed in cpk kidneys (Figure 4, D and E). Serial section analyses of glomeruli from a 10-day-old cpk mouse revealed that 100% of 21 glomeruli were connected by normal-appearing GTJ to proximal tubules (Figure 4F); in contrast, in the terminal stage of the disease (19-day-old cpk mouse), 74% of the glomeruli examined (n = 27) were normal, 22% attached to atrophic GTJ, and 4% were atubular.

Cyst Progression and Proximal Tubular Injury in Human ADPKD Parallel Those in pcy Mice

*Lotus*-stained kidney sections from a patient with early-stage ADPKD, although containing cysts, also revealed abundant proximal tubules with accompanying glomeruli (Figure 5, A–C). In this kidney, regions can be found in which normal-appearing, *Lotus*-stained proximal tubules (Figure 5B) lie adjacent to patches of small, atrophic tubule remnants (Figure 5C). Kidney sections from a patient with advanced ADPKD showed enlarged cysts with scattered, dilated, proximal tubules surrounded by expanded interstitium (Figure 5, D and E). Serial section analyses of glomeruli in ADPKD kidneys revealed alterations in the GTJ, as well as the presence of atubular glomeruli (Figure 5F and Supplemental Figure S1). Atrophic parenchyma and atubular glomeruli were found to a variable extent in all three ADPKD tissue samples examined in this study (Figure 5F). Notably, in patients with early-stage disease (subjects 1 and 2; serum creatinine concentration, <2 mg/dL), 12 of 40 (30.0%) glomeruli examined were attached to atrophic tubules, 7 (17.5%) were atubular, and the remaining 21 (52.5%) GTJs were normal. Kidney tissue from a patient with end-stage disease (subject 3) showed the most severe GTJ damage and loss of proximal tubules, associated with 15 (75%) of 20 glomeruli attached to atrophic tubules, 5 (25%) atubular, and no normal GTJ (Figure 5F). Previously reported patients with isolated proteinuria and normal renal histological features had no glomeruli attached to atrophic tubules, whereas 3 (4.2%) were atubular, and the remaining 68 (95.8%) were normal.12

Discussion

The results of the present study are consistent with the long-held view that expanding cysts diminish or block urine flow in tubules in which they form, as well as compressing and obstructing adjacent tubules. First, cysts block flow in the nephron segments in which they form because careful examination by scanning electron microscopy of cysts in ADPKD kidneys reveals that once cysts reach approximately 2 mm in diameter, they have lost their inlet and outlet connections to become separated from the parent nephron. Second, scanning electron microscopy also clearly demonstrates compression of adjacent noncystic parenchyma. Furthermore, when an inner medullary collecting duct becomes obstructed or compressed, the effect would be magnified because of the resulting obstruction of...
hundreds of upstream nephrons that drain into the blocked duct (Figure 1F). In addition, most cysts eventually disconnect from the tubules in which they derive; consequently, urine flow is blocked completely when the cysts separate from the tubules of origin. In this sense, PKD can be considered a form of piecemeal obstructive nephropathy. This hypothesis is supported by the close correlation of GTJ injury with total fractional cyst area in pcy mice (Figure 2D).

Murine Models of PKD and UUO Point to a Common Mechanism for Nephron Loss through Proximal Tubular Injury

The similarities in the pathological responses to UUO and PKD, reported in 2000, were largely unappreciated until recently. In contrast to acute kidney injury, kidneys do not experience a uremic environment with either UUO or PKD. Recent re-examination of complete UUO in the adult mouse reveals that oxidative stress and massive proximal tubular cell death develop after only 7 days of obstruction, leading to the formation of atubular glomeruli. The proximal tubule, with its rich complement of mitochondria, dependent on oxidative phosphorylation, is the nephron segment most susceptible to oxidative injury. ADPKD patients on the cusp of declining GFR have plasma biomarker levels indicative of oxidative stress [8-epi-prostaglandin F (PGF)2 and superoxide dismutase]. These factors, along with tubular epithelial abnormalities resulting from PKD1 or PKD2 mutations, likely aggravate proximal tubular injury resulting from downstream tubular obstruction as cyst size continues to increase, leading finally to the formation of atubular glomeruli. The expansion of cysts in the pcy mutant mouse follows a pattern similar to that of patients with aggressive ADPKD, and the present morphometric study shows that the fate of the GTJ follows the pattern predicted by UUO in adult mouse kidneys. The slower evolution of GTJ injury in pcy mice, compared with that resulting from complete UUO, is likely the result of sequential partial and complete tubular obstruction in the former. Chronic partial UUO in the neonatal mouse leads to more gradual changes in proximal tubules, and release of obstruction arrests the progression of GTJ injury, with remodeling of the renal architecture. Morphometric studies of the temporal response of the GTJ to chronic UUO in the mouse (Figure 2E) suggest that proximal tubular cell death also accounts for the accelerated loss of functional renal parenchyma and formation of atubular glomeruli in cystic and adjacent compressed nephrons in pcy mice and patients with ADPKD. Consistent with these findings, tubular apoptosis is present in patients with ADPKD and pcy mice, as in mice subjected to UUO. In micropuncture studies in rat kidneys, Evan et al and Tanner and Evan placed wax blocks within...
individual proximal convoluted tubules. Within a week, downstream segments appeared less well differentiated; after a month, they were severely atrophic. Upstream of the blockade, glomerular changes occurred more slowly than in the tubules; after 1 month, glomerular size was decreased and tubules showed evidence of injury that preceded later severe atrophy. These experiments establish that obstruction of solitary proximal tubules leads to severe degenerative changes in segments both proximal and distal to the blockade. Initial anatomical thinning of GTJ and subsequent formation of atubular glomeruli affirm a relentless destructive course during PKD progression. The similarity of these findings to a rat model of ADPKD reported previously, and to the samples of human ADPKD tissue studied herein, indicates that, over time, obstruction of individual renal tubules is likely common to all polycystic renal disorders. Thus, patches of atrophied Lotuss-negative tubules appearing between normal-appearing proximal tubules from a patient with early-stage ADPKD are likely the result of downstream selective nephron obstruction (Figure 5, A and C).

In a new model of ADPKD generated in Pkd1-deficient mice, animals developed increased renal angiotensinogen expression, hypertension, and a urine-concentrating defect in concert with collecting duct cyst development but preserved GFR. This model further reinforces the case for the central role of nephron obstruction in PKD. Immunoreactive renin is markedly increased along afferent arterioles of mice with early-stage ADPKD is likely the result of downstream selective nephron obstruction (Figure 5, A and C).

Formation of Atubular Glomeruli

To what extent does the formation of atubular glomeruli uniquely point to underlying tubule obstruction? Although the glomerulus is secondarily affected by UUO and PKD, injury to the GTJ occurs in a broad spectrum of renal disorders, including diabetes, IgA nephropathy, pyelonephritis, and cystinosis. Although there may be blockage of urine flow in these disorders by proteinaceous concretions, there is mounting evidence in these diseases for oxidative injury to the proximal tubule, leading to GTJ injury and formation of atubular glomeruli. Oliver and Luery recognized the formation of atubular glomeruli in 1935, a finding that has been largely forgotten over time, most likely because of the technical challenges inherent in microdissection or division into serial sections. A recent study of 12 patients with type 2 diabetes (age, 41 to 64 years) revealed 6% atubular glomeruli, 38% glomeruli attached to atrophic tubules, and 56% glomeruli with normal GTJ. In proteinuric patients with type 1 diabetic nephropathy (age, 22 to 47 years), 71% of glomeruli had abnormal GTJ, including 69% atubular glomeruli. The results for patients with ADPKD in the present study are also consistent with progressive injury to the GTJ, which is eventually reflected by increasing serum creatinine concentration. The combined data from clinical studies and animal models suggest that the formation of atubular glomeruli is a terminal remodeling event initiated by a downstream process that blocks urine formation in that nephron, rather than being the direct cause of renal failure.

Early Progression of Cyst Formation in cpk Mice May Model the Course of ARPKD

ARPKD is caused by mutation of PKHD1, which leads to fusiform dilatation of collecting ducts, massive cyst enlargement in utero, and fetal oliguria leading to oligohydramnios. The development of renal failure in ARPKD is highly variable and, unlike ADPKD, cyst growth slows with age. The gene mutated in cpk mice, cystin, is expressed in renal epithelial cilia, and these mice usually die by 3 to 4 weeks of age. As demonstrated in the current study, despite the predominance of massive cysts in the 19-day-old cpk mouse, 74% of GTJs were normal and <5% of atubular glomeruli were present. In contrast to pcy mice, in which spotty cyst formation appears first in the medulla and eventually involves the proximal nephron, proximal tubules show the earliest structural alterations (enlargement, increased mitosis, and necrosis) in cpk mice, and definitive collecting duct cysts do not develop until 1 week of age. Collecting duct cyst enlargement is so rapid in these animals that the anticipated injuries to proximal tubules may not have had sufficient time to become fully expressed as atrophic GTJ and atubular glomeruli.

Evolution of the lesions during the period of early renal maturation is an additional consideration. In mice subjected to UUO within 36 hours of birth, which subjects them to oxidative stress, GTJ integrity is maintained through 14 days. However, after 28 days of UUO, proximal tubules collapse and atubular glomeruli form. This result is likely related to proximal tubular mitochondrial maturation, which renders them less resistant to hypoxia and oxidative injury because of a shift from anaerobic glycolysis to oxidative phosphorylation.

Limitations of this Study

In this study, quantitative histomorphometric techniques previously developed for a murine model of surgical ureteral obstruction were applied in two murine models of PKD, as well as in ADPKD. As shown in Figure 2E, the distribution of normal versus abnormal GTJ in mice subjected to 2 weeks of UUO is similar to that of 30-week-old pcy mice. The evolution of proximal tubular injury after complete UUO is far more rapid than that observed in the pcy mouse. This can be explained by the gradual recruitment of obstructed nephrons as cysts enlarge, with gradations of partial.
obstruction leading to complete tubular occlusion.\^\textsuperscript{17} Although the current study provides inferential support for the obstruction hypothesis, it does not demonstrate causality in individual nephrons. Attempts to demonstrate cessation of tubular fluid flow by injection of filtered markers would be limited by the difficulties in tracing the marker to the point of obstruction in multiple nephrons. Alternatively, injection of inert compounds into the parenchyma to simulate cysts would not replicate the gradual expansion of cysts in vivo. Although data from serial sections are based on a limited number of animals, the fraction of normal GTJ in pcy mice at 9 to 10 weeks and 30 weeks (Figure 2E) closely parallels the fraction of Lotus-positive glomeruli in multiple animals (Figure 2C). This validates the use of Lotus-staining glomeruli in models of PKD as a measure of normal GTJ, as demonstrated previously for the UUO model.\^\textsuperscript{7}

**Conclusions**

We conclude that staggered obstruction of nephrons and collecting ducts contributes to the loss of parenchyma and filtering glomeruli in three types of inherited PKD, including human ADPKD. Cysts can obstruct nephrons and reduce renal function in at least two ways: When a cystic collecting duct separates from the parent tubule, stopping urine flow, potentially hundreds of upstream nephrons that normally drain into that duct are likely to show signs of severe injury, even in the absence of adjacent cysts. Also, cortical and medullary cysts block urine flow more obviously by compressing adjacent normal tubules. In either case, in the early stages of ADPKD, tubule obstruction can account for significant damage to parenchyma in areas lacking cysts. Parenchymal injury may begin early in childhood, long before sufficient glomeruli and tubules have been destroyed to provoke a decline in GFR; the detection of even a few cysts at the earliest stages should be taken as a sign of injury (Figure 1, A and D).\^\textsuperscript{33} For patients with established ADPKD, measurement of total kidney volume is a reliable biomarker of progression,\^\textsuperscript{3} and may serve as a surrogate for nephron loss due to tubular obstruction (Figure 2D). Renal cysts form in utero; therefore, early treatments to slow cyst growth would offer the best chance of limiting GTJ damage caused by the obstruction to urine flow of individual nephrons.

**Supplemental Data**

Supplemental material for this article can be found at http://dx.doi.org/10.1016/j.ajpath.2014.03.007.

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