Why kidneys fail in autosomal dominant polycystic kidney disease

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Abstract | The weight of evidence gathered from studies in humans with hereditary polycystic kidney disease (PKD)1 and PKD2 disorders, as well as from experimental animal models, indicates that cysts are primarily responsible for the decline in glomerular filtration rate that occurs fairly late in the course of the disease. The processes underlying this decline include anatomic disruption of glomerular filtration and urinary concentration mechanisms on a massive scale, coupled with compression and obstruction by cysts of adjacent nephrons in the cortex, medulla and papilla. Cysts prevent the drainage of urine from upstream tributaries, which leads to tubule atrophy and loss of functioning kidney parenchyma by mechanisms similar to those found in ureteral obstruction. Cyst-derived chemokines, cytokines and growth factors result in a progression to fibrosis that is comparable with the development of other progressive end-stage renal diseases. Treatment of renal cystic disorders early enough to prevent or reduce cyst formation or slow cyst growth, before the secondary changes become widespread, is a reasonable strategy to prolong the useful function of kidneys in patients with autosomal dominant polycystic kidney disease.

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Introduction

In contrast to idiopathic conditions, the hereditary polycystic kidney diseases (PKDs) present researchers searching for mechanistic causes with an advantage, as they carry the etiology of the specific condition encrypted within mutated genes. The genes associated with the most common cystic disorders have been identified and decoded, identifying proteins with affinity for diverse sites within renal tubule plasma membranes, nuclei and endoplasmic reticulum as well as the cilia and centrosome apparatus.[1,2] Owing to the complex interplay among these cellular elements, renal cysts develop in association with tubule basement membrane thickening and blossoming interstitial inflammation and fibrosis that will grotesquely scar the renal architecture and eventually undermine renal function. In patients with autosomal dominant PKD (ADPKD), cysts develop in a minority of the renal tubules,[3] leading to a fundamental question: how do so few cysts devastate the function of so many nephrons?

Over the past 20 years, a staggering volume of research has been published elucidating the molecular and cellular mechanisms that bring about the cystic disease phenotype. Yet we remain unsure about which mechanistic breakdown, and the secondary networks of disarray that breakdown provokes, is the primary factor that should be selected for elimination by targeted therapies. Taking into account the severe anatomic distortion exhibited by these kidneys, the cysts are frequently blamed for causing renal failure, although a clear explanation of how this might occur has not been forthcoming. Are cysts the offending agents or is something else responsible for driving the loss of renal function in patients with ADPKD? The development of interstitial changes juxtaposed to cysts[4,5] alerted early researchers to the possibility that an interstitial fibrotic mechanism might initiate the secondary formation of cysts in patients with ADPKD, similar to the way acquired cystic kidney disease (ACKD) in patients with chronic glomerulonephritis and diabetic nephropathy[6,7] develops in a tangle of fibrosis at the end stages of most chronic progressive renal disorders. In this Review, we analyze the available facts pertaining to the development of renal failure in patients who have ADPKD, with a view to determining the respective effects of the cysts and the interstitial changes associated with them on the decline in glomerular filtration rate (GFR).

The clinical course of renal insufficiency

ADPKD is often diagnosed upon the discovery of hypertension, the sudden onset of renal pain or hematuria, or the inadvertent discovery of nephromegaly on physical or radiological examinations.[8,9] Renal insufficiency can appear at any age; however, in the vast majority of patients the initial awareness of renal dysfunction is delayed beyond their fourth decade until estimated GFR declines to a level that is clearly abnormal.[10–12] In the late stages of the disease, the kidneys are massively enlarged and laden with thick bands of interwoven fibrotic tissue sequestering the remaining functioning parenchyma within scattered islands surrounded by cysts (Figure 1).[13,14]

Competing interests
J. J. Grantham declares an association with the following company: Otsuka Pharmaceutical Group. See the article online for full details of the relationship. The other authors declare no competing interests.

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Key points

- In patients with autosomal dominant polycystic kidney disease (ADPKD), cysts develop in a minority of cortical and medullary tubules, enlarge exponentially and compress adjacent normal parenchyma
- Individual expanding cysts displace normal tubules, blood vessels and lymphatics, obstruct their flow and promote apoptosis, atrophy and fibrosis of functioning parenchyma
- Owing to dichotomous branching of the ureteric bud, cysts forming in medullary collecting ducts will probably impair the function of more upstream nephrons than cysts developing in the cortex
- Despite the pernicious loss of functioning nephrons, glomerular filtration rate (GFR) does not decline until fairly late in the course of ADPKD, probably because of compensatory hyperfiltration in surviving nephrons
- In the third to fourth decades of life, spreading nephron obstruction, together with interstitial inflammation and fibrosis, widens the devastation of residual parenchyma, promoting the inexorable loss of renal function
- Potential therapies that target cyst growth mechanisms and are initiated early in the disease course (stage 1 chronic kidney disease) will probably be more effective than treatments starting after GFR has declined

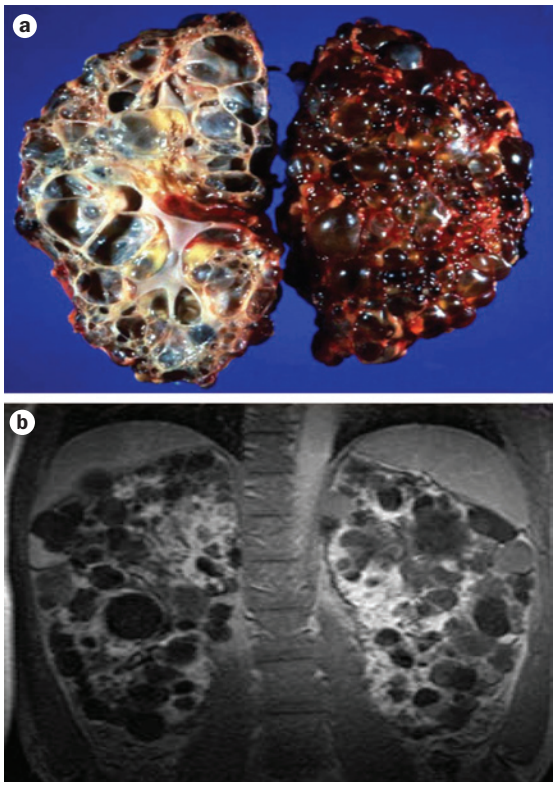

Figure 1 | End-stage polycystic kidneys. **a** | Cut section and surface features. Cysts are separated by fibrotic bands leaving no visible parenchyma. Permission obtained from T. I. Steinman, Beth Israel Deaconess Medical Center, Harvard Medical School, MA, USA. **b** | Similar kidneys examined by gadolinium-enhanced T1 MRI. Gadolinium is filtered and concentrated by the tubules, which makes some residual parenchyma, shown in white, stand out among the cysts. The focal areas of gadolinium enhancement identifies tissue that continues to function.

The development of hypertension, microscopic or macroscopic hematuria, nephrolithiasis and renal pain are signs of notable renal injury that occur long before the GFR declines. Glomerular hyperfiltration,[15] loss of urine concentrating capacity[16] and mild albuminuria[17] might be premonitory signals that the functioning renal parenchyma is under stress. Results from contrast-enhanced MRI[18,19] and CT[20–22] scans indicate that functioning renal tissue might be lost many years before the actual decline in GFR can be verified with certainty. Highly efficient compensatory hyperfiltration by glomeruli serving noncystic tubules seems to maintain the GFR within a normal range despite the massive buildup of cysts and the loss of functioning parenchyma.[10] The extent to which this hyperfiltration might be harmful to the overworked glomeruli and tubules is unknown. Longitudinal studies reveal that in most patients with PKD1, and nearly all patients with PKD2, the GFR remains within a range that is clinically difficult to distinguish from normal, and then begins an unrelenting descent that reaches an average decline of ~5 ml/min/1.73 m^2/year and culminates in end-stage renal disease (ESRD).[10,12,15,23]

So what is of primary importance in the progression of renal insufficiency in ADPKD? Is it the collective mass of expanding intrarenal cysts that causes kidney function to fail or is it extracystic factors, for example extracellular matrix and inflammatory processes. Or is a combination of effects required?

Pathogenetic components

Initial formation and growth of renal cysts

Epithelial cysts are fairly simple multicellular structures that occur within complex, converging arcades of tubules engaged in the formation of urine. To be considered a cyst, a dilated tubule segment must exhibit more mural cells than could be accounted for in a normal tubule of identical length. Increased transtubule pressure will dilate a tubule; however, the elastic basement membrane limits that distension to a diameter <100 µm in mammalian tubule segments.[24] Cortical tubules that are sectioned at right angles to the longitudinal axis appear circular in plane projection and contain ~6–8 circumferential cells.[25] Thus, in histological sections, circular tubule segments larger than ~100 µm in diameter that contain >12 circumferential cells are likely to be cystic.

The number of nephrons in normal human kidneys ranges between 400,000 and 1,200,000,[26] and a minority of these develop cysts in patients with ADPKD. The cysts are scattered throughout the renal medulla and the cortex in adult kidneys, occasionally appearing to 'sprout' from the surface (exophytic cysts).[27] Asymmetric development between kidney pairs occurs, but is rare, and most patients experience fairly equal bilateral renal enlargement.[18]

In scanning electron micrographs, the number of cells in cyst walls greatly exceeds that found in normal tubules, which implies that focally increased epithelial cell proliferation must have occurred to account for the increase in cell number.[3] Importantly, however, lumen volume accounts for most of the space within a cyst and the rate of cell proliferation in the single layer of epithelial cells lining cyst walls is slower than might be observed in solid malignant tumors of equivalent size or in cells growing in culture. Biochemical markers of

proliferation[28–30] are often expressed in the cyst epithelial cells at levels only modestly greater than that of terminally differentiated renal tubules. Despite the fairly slow rate, cell proliferation underlies the progressive increase in total kidney volume that averages ~5–6% per year.[18,19]

The fluid that accumulates within the cysts derives from two potential sources: unreabsorbed glomerular filtrate and transepithelial solute and fluid secretion.[3,31] As long as the tubule remains functionally connected to the cyst, unreabsorbed glomerular filtrate will flow into the cyst and the low levels of transepithelial fluid secretion will be masked by the larger volume of glomerular filtrate flowing downstream into the renal pelvis. However, the majority of cysts separate from the parent tubule to become isolated sacs: only about one-quarter of the cysts in adult kidneys remain connected[3] and the fluid within them exhibits electrolyte and pH values typical of collecting duct urine.[32,33] In addition, evidence indicates that most of the cysts in adult patients with ADPKD have biomarker features of collecting ducts.[34] Isosmotic transepithelial secretion of chloride is the principal mechanism for adding solutes and fluid to the lumen.[35] Even though the rates of chloride secretion into the isolated cysts are reasonably slow, they are greater than the rates of solute absorption and, therefore, fluid progressively accumulates within the cyst.

Cellular and tubule responses

In patients with ADPKD, a cyst is formed in a renal tubule when focal epithelial cell proliferation provokes radial expansion to form a sac-like protrusion out of the tubule segment (Figures 2 and 3). A phenotypic conversion within a tubule epithelial cell commits it to the formation of a cyst rather than an elongating tubule. Although the altered cells generate proteins found in immature segments, within the cyst wall they retain molecular and functional features of terminally differentiated tubule segments, linking them to the parent nephron.[36] Cysts within glomerular capsules, proximal tubules and loops of Henle are seen at the earliest stages of ADPKD, but during the later stages these cysts diminish in abundance and cysts in the collecting ducts supervene. Morphologic features that distinguish principal collecting duct cells from intercalated collecting duct cells are frequently lost as the patient ages and the cysts expand. The epithelium within the cysts generates chemokines, cytokines, angiogenic factors, interstitial collagens and other matrix proteins (Box 1). The renal expression of cell proliferation and apoptosis markers is consistent with increased cellular turnover in cystic tubules, neighboring noncystic tubules and interstitial cells.[37–43] Transepithelial electrolyte transport polarity is retained in cysts derived from collecting ducts.[44] Salt secretion driven by chloride, which is ordinarily obscured in normal tubules by aggressive solute reabsorption, fills the lumens of the isolated sacs with fluid.[45,46]

Arginine vasopressin (AVP), through cyclic AMP, leads to chloride secretion into the cysts and promotes increased proliferation of the lining cells.[36] For all practical purposes the human kidney operates under an 'AVP clamp', which ensures the excretion of urine that is more concentrated than the plasma throughout the day and night. Thus, AVP continuously promotes cellular proliferation and fluid secretion by cysts and is a dominating factor that controls the rate of cyst and kidney enlargement in patients with ADPKD and other hereditary cystic disorders.[36,47–49]

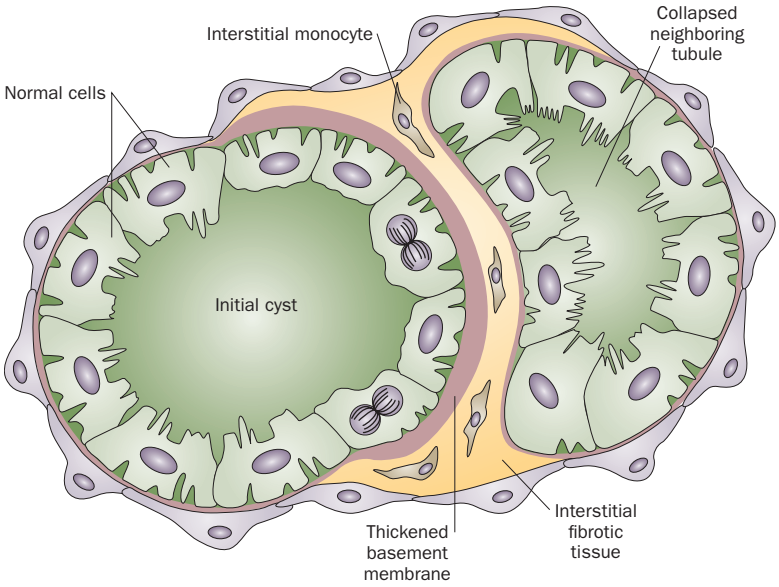

Figure 2 | Schematic illustration of the early stages of cyst formation and enlargement. A tubule cell divides repeatedly generating less well differentiated daughter cells that extend the tubule wall. Excess tubule basement membrane material is deposited beneath the altered mural epithelial cells defining the nascent cyst. Mononuclear cells (macrophages and fibroblasts) and collagen fibrils appear in the interstitium subjacent to the evolving cystic portion. The cyst expansion compresses adjacent tubules and, though not shown here, compromises capillaries, lymphatics and larger caliber arterioles and venules. Permission obtained from Science and Medicine © Grantham, J. J. *Science and Medicine* **9**, 128–139 (2003).

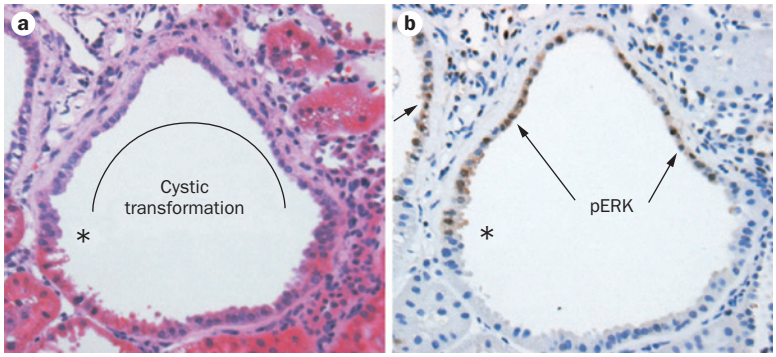

Figure 3 | Early stage cyst formation. **a** | ADPKD in a Han cy/+ rat. Half-cyst and half-tubule section stained with hematoxylin and eosin, which shows the sharp transition between the cyst and adjacent tubules (asterisk). Note the thickening of the tubule basement membrane and extracellular matrix beneath the area labeled 'cystic transformation'. **b** | Immunohistochemical staining of an antibody to pERK (arrows) exclusively in the cystic portion of the hemi-cyst shown in part a. Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; pERK, phosphorylated extracellular signal-regulated kinase. Permission obtained from Nature Publishing Group © Nagao, S. *et al. Kidney Int.* **63**, 427–437 (2003).

Box 1 | Factors common to cystic diseases and UUO

- Basic fibroblast growth factor*[70,73]
- Clusterin*[70,71,73]
- Collagen I, III, IV, V, VI*[5,85,96]
- C-reactive protein*[73,77]
- Epidermal growth factor receptor*[70,73]
- IL-1, IL-2, IL-6 and IL-8*[5,81,83]
- Kidney injury molecule 1*[84]
- Membrane metalloproteinase 2*[80]
- Monocyte chemotactic protein 1*[83,85]
- Neutrophil gelatinase-associated lipocalin*[84]
- Nuclear factor κB*[70,73]
- Osteopontin*[73,85]
- Prostaglandin E2*[73,81]
- Renin–angiotensin–aldosterone*[58,70,74]
- Smad 2/3*[73,96]
- Superoxide dismutase‡[70,71,73,77]
- Tumor necrosis factor*[81,83]
- Transforming growth factor β*[81]
- Vimentin*[70,73]

*These pathogenetic factors are raised in renal cystic diseases and UUO. ‡This pathogenetic factor is decreased in renal cystic diseases and UUO. Abbreviation: UUO, unilateral ureteral obstruction.

A seminal study of human autosomal recessive PKD (ARPKD) in a rat ortholog (PCK; *Pkhd1*$^{-/-}$ rats) indicated that a specific growth factor, in this case AVP, might be required in addition to mutated genes to initiate cyst formation in collecting ducts.[50] *Pkhd1*$^{-/-}$ animals were crossed with Brattleboro rats (AVP$^{-/-}$) to develop animals lacking *Pkhd1* and AVP. Not only did double-null pups (*Pkhd1*$^{-/-}$ AVP$^{-/-}$) have kidneys that were remarkably smaller than *Pkdh1*$^{-/-}$ AVP$^{+/+}$ animals for as long as 20 weeks after birth, they did not develop notable numbers of cysts over the course of the study. By contrast, when 12-week-old double-null animals were administered with desmopressin (DDAVP® [Ferring, The Netherlands]) by osmotic mini-pump, countless cysts and enlarged kidneys developed by 20 weeks of age that were equal to or greater than those of the animals with regular PCK (Figure 4). Double-null animals that did not have renal cysts at 20 weeks of age had blood urea nitrogen levels and renal fibrosis scores equal to those of wild-type normal rats, whereas those that received desmopressin had azotemia and renal fibrosis equivalent to the ordinary PCK rats.

Importantly, this study revealed that increased cellular generation of cyclic AMP was necessary to initiate cyst formation as well as to promote their growth in animals carrying two mutated *Pkhd1* alleles. This finding is unambiguous evidence that another 'hit', in this case AVP, in addition to the mutation of both alleles, is required to stimulate cyst formation. Moreover, interstitial inflammation and fibrosis did not develop in the animals lacking *Pkhd1* and AVP, indicating that mutated genes alone were incapable of generating the disastrous consequences of unbridled ARPKD.

In humans, tubule epithelial cells bearing PKD1 or PKD2 germline mutations evidently acquire the second and third 'hits' required for the formation of cysts *in utero*, as some cysts begin to proliferate in that growth-factor-rich environment.[51] At ~9 weeks gestation, the growth rates of tubule epithelial cells increase in some segments to promote unqualified cystic dilations, as identified in microscopic sections of fetal kidneys.[51] However, identifying single renal cysts during pregnancy on a large scale is problematic, as the threshold for ultrasound detection in skilled hands requires a cyst diameter >5 mm.[52] Although unusual, babies with very enlarged kidneys attributable to ADPKD might be misdiagnosed at birth as having ARPKD. The presence of these massively enlarged kidneys at birth in both genetic disorders proves that cysts might begin to form and enlarge *in utero*. Children whose kidneys are not massively enlarged probably escape detection at birth, as ultrasound scans are not done routinely. The molecular basis of the very early onset of huge cystic change is unknown, although the number of cysts that form *in utero* is probably a major determinant. Thus, the formation and enlargement of renal cysts *in utero* might be more frequent than generally appreciated.

The age at which cysts form is a critical issue for understanding the pathogenesis of ADPKD, as evidence indicates that they enlarge progressively at a fairly constant rate in children[53] and adults[19,54] with ADPKD. The earlier in life a cyst is formed the longer it has to grow. If most of the cysts form *in utero* and grow at accelerated rates in the fetus they would become the largest cysts in the newborn infant and possibly hold that distinction for the rest of the patient's life.[55]

Do cysts continue to form during the lifetime of a patient? In the Consortium for Radiologic Imaging Studies of PKD (CRISP) in which MRI scans were used to assess adult patients, the number of renal cysts increased over a 3-year period.[56] However, it is not known if newly recognized cysts developed *de novo* or were simply below the limit of detection (~2 mm diameter) in the baseline study.[52] In a preliminary examination of parenchyma in four adult nephrectomy specimens from patients with early stage ADPKD, the authors found cysts with diameters <2 mm in all the specimens, proving that cysts are not always visible using the most sensitive imaging methods. However, we do not know if these cysts were newly formed or simply stragglers from the *in utero* or neonatal experience. Finding small cysts among the large cysts does not necessarily mean that they arose in response to the same mechanisms that caused the ADPKD. As renal function declines, forces develop that can promote the formation of renal cysts in individuals without mutated cystic disease genes; however, acquired cystic kidney disease (ACKD) usually occurs in severely fibrotic kidneys at the end stages of the primary renal disorder.[57]

Effects of cyst mass

One thing that gets your attention when examining an end-stage polycystic kidney for the first time is how

unattractive they are (Figure 1). At the end stage of disease there can be little doubt that cysts infringe upon and compress everything nearby, including the pelvic collecting system, arteries, veins, microvasculature, lymphatics and what is left of the functioning parenchyma. The delicately tuned glomerular filtration, glomerulo–tubule feedback and medullary countercurrent mechanisms are wrecked by anatomic distortions. Vascular path lengths for intrarenal perfusion are extended and the vessels are narrowed by stretching, which probably contributes to increased renovascular resistance and reduced blood flow.

The development of hypertension in children with ADPKD unveils the mass effect of cysts to reduce vascular perfusion and locally activate the renin–angiotensin–aldosterone system.[53,58–61] Patients are also highly susceptible to intrarenal and perinephric hemorrhage and urinary tract bleeding secondary to fairly minor abdominal trauma. Cyst expansion within the kidney invariably forces anatomic accommodation by adjacent tubules, microvessels and interstitium (Figure 5a). The distending pressures within these tumorous masses might be more than three times higher than in the adjacent tubules, sufficient to cause partial or complete obstruction.[3,33,62] Lumen compression by taut cysts seems to slow the fluid flow through tubules, blood vessels and lymphatics (Figure 5b–e). Results from MRI scans demonstrate hyperconcentration of gadolinium in tubules adjacent to cysts, consistent with the reduction of urine flow through narrowed lumens. The most economical interpretation of this finding is that the resultant increased fractional reabsorption of glomerular filtrate within the squashed tubules increases the lumen concentration of impermeant gadolinium, which creates bright stains adjacent to black cysts in MRI scans.

The pathophysiology of nephron obstruction

Cyst formation in ADPKD kidneys most often exhibits a generalized pattern, although superficial cortical and deep medullary distributions are occasionally seen (Figure 6a). Cysts can interfere with the flow of urine by separating from the tubule of origin, thereby stopping urine formation in a single unit, and by compressing adjacent parenchyma. Cyst formation in a nephron that extends unbranched from the glomerulus to the cortical collecting duct would remove only that unit from the overall production of urine. However, if there are tubules draining into the segments upstream of the cyst the overall effect of partial or complete obstruction to urine flow within a solitary cyst can be magnified. About 44 collecting ducts in each papilla drain urine into the renal pelvis and each collecting duct drains ~2,800 upstream tubule segments.[63] Consequently, formation of a cyst in a single papillary collecting duct would potentially impede the contributions of ~2,800 upstream segments to the final urine. Cysts forming higher up in the collecting duct system would have a progressively reduced collateral effect. The cortical collecting duct, the last segment of the collecting duct system, receives urine from ~11 nephrons.

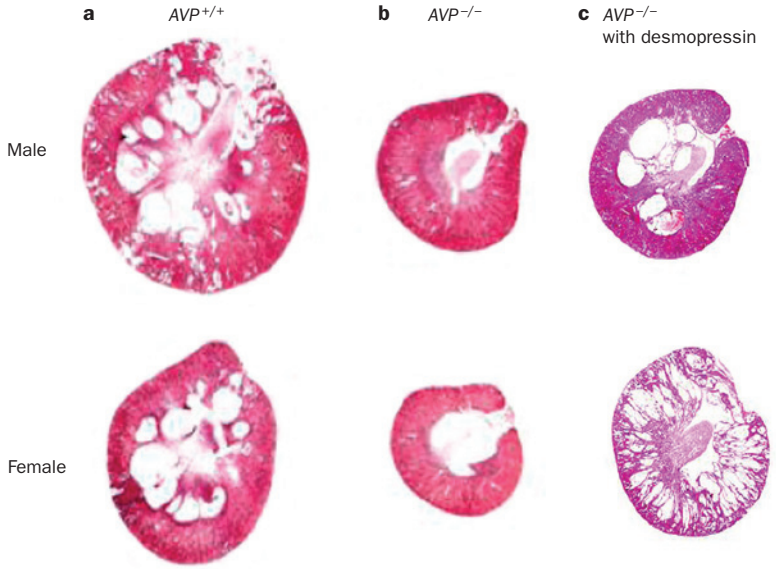

Figure 4 | Effect of removing AVP on the development and growth of cysts in *Pkhd1* rats (autosomal recessive polycystic kidney disease ortholog). **a** | In the absence of the *Pkhd1* gene product, cysts developed in the outer medullary and cortical collecting ducts of male and female animals shown at 20 weeks of age. **b** | In the complete absence of AVP, the kidneys appeared normal except for a fairly large renal pelvis secondary to the large urine flows of diabetes insipidus. **c** | Administration of desmopressin by osmotic mini-pump for 8 weeks caused cysts to form in medullary and cortical collecting ducts in both male and female animals. Abbreviation: AVP, arginine vasopressin. Permission obtained from American Society of Nephrology © Wang, X. *et al. J. Am. Soc. Nephrol.* **19**, 102–108 (2008).

In addition to blocking the tubule segment in which the cysts formed, the expanding masses can slow the flow of blood, lymph and urine in the vessels and tubules adjacent to them. Medullary cysts can also have a more serious overall potential effect than do cortical cysts (Figure 6b,c). For example, a cyst that is 400 μm in diameter—below the limits of current radiologic detection—has the potential to block at least 32 tubules adjacent to it in the cortex (Figure 6b). By contrast, a cyst of the same diameter in the inner medulla that deletes or compresses six collecting ducts has the potential to diminish urine flow from ~16,800 upstream tubules! Moreover, cysts are 3D structures; therefore, as the cysts expand they exponentially engage and compress neighboring renal tubules and microvessels, which reduces flow through them. The effect on overall renal blood flow and urine formation will be magnified in patients who develop reasonably large numbers of cysts, that is, higher in PKD1 versus PKD2,[56] perhaps explaining in part why patients with PKD1 reach ESRD nearly 20 years before patients with PKD2.[64–66]

Animal models support a role for obstruction

The Han cy/+ rat, a model of PKD, caused by a spontaneous mutation of *Anks6*,[67] has no human counterpart as far as we know, but has a clear autosomal dominant mode of inheritance and a clinical course similar to human ADPKD. Large numbers of cysts form primarily in proximal tubules and lead to renal failure within a year. Partial and complete obstructions to urine flow in

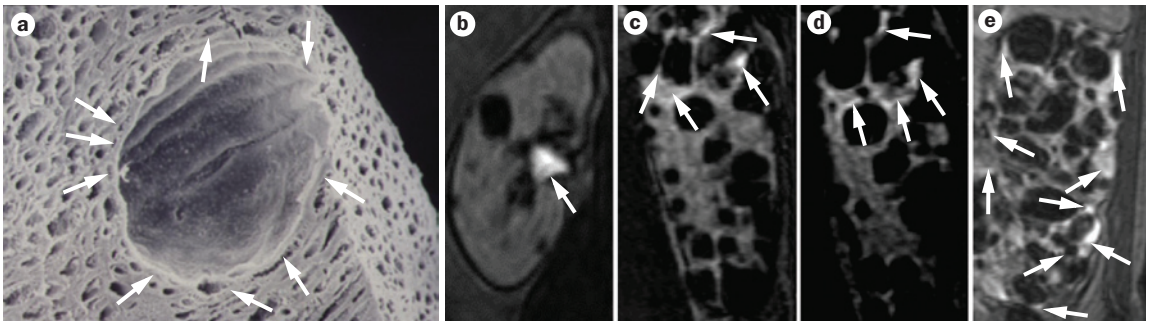

Figure 5 | Evidence of tubule obstruction by cysts. **a** | Scanning electron micrograph of a solitary cortical cyst surrounded by normal parenchyma in a patient with early-stage autosomal dominant polycystic kidney disease. The cyst in cross-section (diameter ~500 μm) crowds neighboring renal tubules (arrows), which potentially reduces the rate of urine flow through them. **b** | T1 MRI scan of a right kidney in a patient with mild disease showing a few cysts surrounded by normal parenchyma that appears brighter than the background structures. Gadolinium distributed throughout the parenchyma is in contrast to the black holes denoting cysts. The renal pelvis (arrow) displays gadolinium intensity even greater than in the parenchyma. **c**–**e** | In patients with more advanced disease, there is evidence of pericystic compression of adjacent renal tubules in T1 MRI scans after intravenous infusion of gadolinium, as in those tubules the scanning agent is concentrated to levels higher than levels in the adjacent parenchyma. **c** | A left kidney that shows focally bright gadolinium enhancement greater than parenchyma adjacent to cysts (arrows) in the upper part of the kidney. **d** | From the same kidney, 3 mm anterior showing continuity of hyperdense gadolinium collections. **e** | A left kidney from another patient with more advanced disease showing more widespread hyperdense gadolinium collections.

single superficial proximal tubules are frequent and early events in 2–4-month-old Han cy/+ males.[68]

The injection of wax into the proximal tubules of normal rats to completely obstruct urine flow caused changes that resulted in tubule cell atrophy in the downstream segment beginning a few days after urine flow was blocked.[69] By contrast, in the segment proximal to the obstruction, tubule morphology remained intact for a few more days before atrophy intervened. Thus, focal obstructions to urine flow cause atrophy of the blocked nephrons in due course. The response of renal tissue to unilateral ureteral obstruction and PKD are similar.[70] In simple unilateral ureteral obstruction the renal tubules of rats and mice initially dilate followed a few days later by processes culminating in atrophic and fibrotic parenchyma surrounding a dilated pelvic sac.[71,72] Obstructed kidneys result in a rich array of chemokine, cytokine, paracrine and autocrine products,[72,73] many of which are also produced within cystic kidneys (Box 1). Thus, piecemeal urinary obstruction in PKD seems to result in some of the same futile renal repair mechanisms that occur on a larger scale in response to unilateral or bilateral ureteral blockade.

Vascular–interstitial–inflammatory responses

In patients with advanced disease, the renal vasculature is radically distorted and even in young patients with small kidneys the cysts are associated with the development of hypertension, renal pain and hematuria, reflecting their propensity to cause renal injury.[54,60] The stretching of intrarenal arteries and arterioles and the obstructed urine flow of renal tubules both contribute to the increased production of renal renin that activates the vasoconstrictor angiotensin II.[74] Reduced renal blood flow seems to precede the decline in GFR by several years,[75] setting up opportunities for regional hypoxia and cellular injury shown by increased expression of hemeoxygenase[76] and exacerbated by decreased levels of superoxide dismutase.[77]

In contrast to the situation in the kidneys, the massive formation of cysts within the livers of patients with ADPKD has no adverse effects on hepatic synthetic or excretory functions.[78] The fact that cyst formation and enlargement is more problematic in kidneys than in livers is probably attributable to the elaborate vascular–tubule–lymphatic architectures required to generate a glomerular filtrate in the renal cortex and to concentrate urine in medullary collecting ducts, neither of which tolerate being misshapen. In addition, the liver has a regenerative capacity that can augment its secretory and synthetic functions, which the kidneys are not able to do.

The relationship between cyst formation and the development of interstitial inflammation and fibrosis is highly variable in ADPKD. A careful study of the Han cy/+ rat identified tubules in the initial stages of cyst formation and found that phosphorylated extracellular-regulated kinase (pERK), which is implicated in the growth of human renal cysts, was expressed in cells that had undergone cystic transformation (Figure 3).[79] At the junctions between the transformed and the normal tubule cells there was an abrupt transition in the extracellular matrix and tubule basement membrane beneath the cyst cells. As the basolateral surface of normal tubules and the extracellular matrix adjacent to it exhibited no abnormalities it stands to reason that the tubule basement membrane and matrix changes evolved as epithelial cell proliferation created the cystic outcropping.

A striking feature of the end-stage polycystic kidney, apart from its size, is the abundance of dense fibrotic bands of collagen and other interstitial matrix components outlining the cysts (Figure 1). In patients with moderately or far advanced disease, renal arterioles exhibit intimal thickening, smooth muscle hypertrophy and global, but not focal, glomerular sclerosis.[14] Tissue

ischemia activates the local production of angiotensin II, further contributing to a broad scale of injury that compromises renal viability and function. Metalloproteinases are activated that further modify the structure of the extracellular matrix.[80]

Chemokines and cytokines, many of which are also produced as a result of unilateral or bilateral ureteral obstruction (Box 1), accumulate within the cysts and seem to derive in large measure from the cyst epithelial cells.[81–84] Mononuclear cells, including macrophages and fibroblasts exhibiting smooth muscle actin, invade the renal interstitium to create a low-grade tubule–interstitial reaction.[14,85] As noted above, localized interstitial inflammation and fibrosis around cysts apparently begin fairly early but do not become a pathologic feature until reasonably late in the disease course. This pattern is in contrast to the one seen in ACKD, where the cysts develop rather late in the masses of fibrotic matrix generated in response to the primary disorder.[86]

Do cysts or interstitial changes come first?

The striking lamination of basement membrane material observed in the initial electron micrographs of the basolateral surface of human renal cysts raised the possibility that the extracellular structure was unable to support pressurized renal tubules.[4] Wider examination of human ADPKD shows that cyst basement membranes might also be thickened or appear entirely normal. Nagao's study in the Han cy/+ rat provided an early examination of evolving cysts that supported a tight linkage between the abnormal extracellular matrix structures and the formation of the cyst.[79] Shannon *et al.*[87] generated a hypomorphic mutation in the mouse laminin α5 gene that caused cysts to form in all tubule segments, which led to death from renal failure in 4 weeks. A study in zebrafish embryos published in 2010 revealed that orthologs of human polycystins regulate extracellular matrix secretion or assembly and—when mutated—could lead to altered matrix integrity, which might cause an aberrant response from the tubule epithelial cells.[88]

By contrast, several studies in mice with *Pkd1* or *Pkd2* mutations failed to find visible changes in the tubule basement membrane or extracellular matrix in fetal kidneys or newborns,[30,44,89–96] whereas in later stages of the disease, tubule basement membrane and interstitial changes were abundant.[30,91,92,94,96,97] More to the point, cysts formed in collecting duct principal cells of mice following selective deletion of *Pkd1*,[95] illustrating that if altered extracellular matrix secretion or assembly are involved in cystogenesis they must be mediated by the tubule epithelial cells from which the cysts arise rather than by external sources. Thus, the evidence is consistent with cyst formation beginning within tubule cells. At the same time, the newly minted and variably dedifferentiated cyst epithelial cells produce aberrant tubule basement membranes and generate chemokines, cytokines and growth factors that cause macrophages and fibroblasts to wage the devastation culminating in fibrosis. The expression of some of these combatants is unique, in comparison with terminally differentiated cells in neighboring renal tubules.[82] Seemingly, the transformation from a normal tubule to a cyst elicits a futile repair response that persists as the unusual neoplasm progressively enlarges.

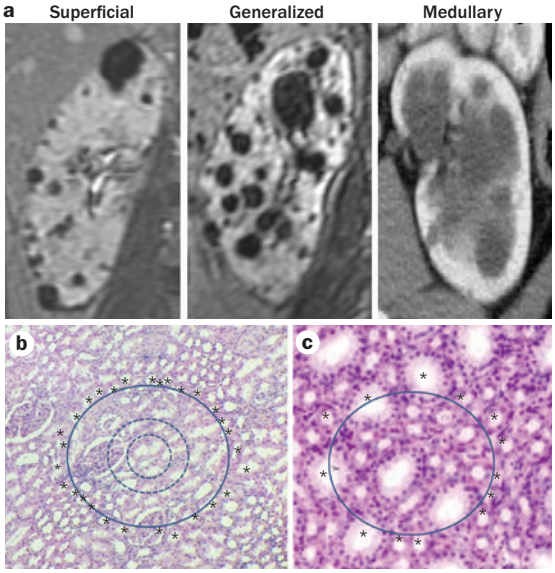

Figure 6 | Topography of renal cyst formation in ADPKD. **a** | Patterns of renal cyst formation in ADPKD. T1 MRI scans with gadolinium enhancement of parenchyma illustrating cyst formation principally in superficial cortex (left), generalized (middle) and medullary (right) distributions. Most patients exhibit the generalized pattern. **b** | Cysts encroach on increasing numbers of tubules and glomeruli as they expand. A cross-section of a normal kidney cortex illustrates the increasing pericystic effect on neighboring parenchyma of a solitary cyst as its diameter grows from 100 μm to 200 μm to 400 μm. Asterisks identify adjacent tubules ($n = 29$) that would be directly impacted by a cyst 400 μm in diameter. In addition to external compression, 36 tubules would be displaced by the cyst. **c** | Cross-section of normal renal medulla illustrating the potential pericystic effect on adjacent renal tubules of a cyst 400 μm in diameter. Asterisks identify adjacent tubules touched by the cyst; 13 in total (four collecting ducts, nine limbs of the loop of Henle) displacing 13 tubules (two collecting ducts, 12 loops of Henle). Hematoxylin and eosin stains were used. Abbreviation: ADPKD, autosomal dominant polycystic kidney disease.

Timing of cell proliferation in cystogenesis

In dominantly inherited disease, the cysts develop as saccular outpouchings of a tubule segment[79,98] (Figure 2), perhaps reflecting clonal growth.[99] By contrast, in ARPKD the cysts usually take on a cigar-shaped configuration, as several contiguous collecting duct cells participate in the expansion process.[50,79,100,101] Studies have clearly shown that in dominant and recessive disorders mutations in both tubule cell alleles do not guarantee that a cyst will evolve,[50,102] inferring that another factor is necessary to start the process. Such a third 'hit' can apparently be supplied as a result of acute kidney injury.[103]

In adult mice in which *Pkd1* is inducibly knocked out, cyst formation occurs variably and slowly over time. However, ischemia–reperfusion kidney injury, which

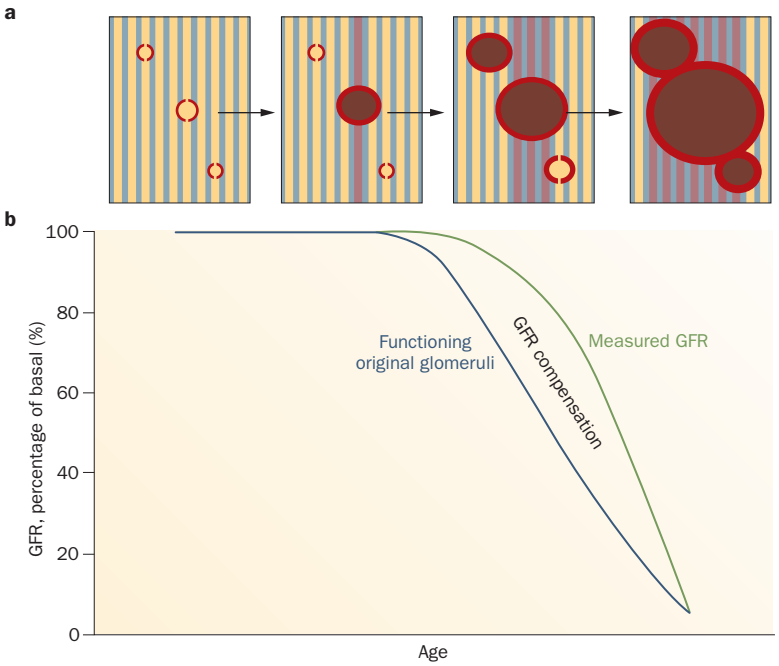

Figure 7 | How cysts decrease GFR. **a** | Schematic of renal parenchyma showing tubules (yellow) and evolving cysts with thickened tubule basement membrane and early fibrosis (red circle). Expanding cysts that are disconnected from parent tubules (brown) negate functional contributions by their afferent and distal segments (pink) that are irretrievably lost (by atrophy or apoptosis) and replaced by fibrosis. Moreover, physical displacement and compression of neighboring normal tubules reduces their function as well, which leads to pericystic fibrosis (red). **b** | GFR in relation to physical changes within the kidneys. Measured GFR (green), expressed as a percentage of the expected basal function for that age, remains within normal limits for an extended period before entering a late period of increasing decline to renal failure. Age is not defined to allow for the variable ages when the GFR decline might be detected in individual patients. The percentage of functioning original glomeruli (blue) declines before measured GFR falls owing to compensatory hyperfiltration of residual functioning glomeruli, assumed here to ultimately double their basal single-nephron GFR for age. As fully compensated nephrons also fail, total GFR becomes inadequate to maintain life. Late in the disease, hypoxia, inflammation and fibrosis contribute to declining GFR, independent of cyst enlargement. Abbreviation: GFR, glomerular filtration rate.

promotes tubule epithelial cell proliferation, rapidly induces cyst formation.[29,104,105] Likewise, PCK/Brattleboro rats lacking *Pkhd1* and vasopressin do not form cysts in the collecting ducts unless the hormone is administered and cell proliferation is activated. This finding implicates hormones with mitogenic activity towards renal epithelial cells as candidates for the second 'hit' that is necessary for the development of ADPKD (Figure 4). These studies and others[3,89–91,97,105] support the view that cell proliferation is instrumental in the formation and the continued enlargement of renal cysts.

Pathogenesis of renal insufficiency

Cystogenesis apparently begins when the levels of standard polycystin 1 or polycystin 2 are reduced below the threshold required to maintain orthodox tubule geometry; however, loss of polycystin function alone is evidently not sufficient to form a cyst. Unidentified mitogens promote proliferation of the mutated cells, which causes a saccular cyst to emerge as the basement membrane and extracellular matrix are modified and give way to the expanding mass. In a failed attempt at injury repair, epithelial cells comprising the nascent cyst produce chemokines and cytokines that promote inflammation and fibrosis in the adjacent interstitium as intraluminal pressure presses the expanding masses against surrounding lymphatics, capillaries and unaffected tubules (Figure 2). Macrophages, called into the kidneys, together with resident fibroblasts and fibroblasts that have undergone epithelial–mesenchymal transformation weave a network of fibrotic curtains in a fruitless attempt to contain the perceived injury.

The cysts remain attached to the parent tubule until they reach a diameter >2 mm, at which point about three-quarters of cysts separate to become isolated sacs that fill with fluid exclusively by transepithelial secretion. Obstruction by cysts of otherwise normal tubules leads to additional chemokine and cytokine production and widespread tubulointerstitial fibrosis. Obstruction of capillaries and arterioles affects glomerular filtration and perfusion of the countercurrent multiplier capillaries, reducing the kidney's capacity to produce maximally concentrated urine.

As cysts enlarge, these disruptive processes are repeated endlessly, renal parenchymal integrity is further compromised and the efficiency of the compensation for a reduced GFR decreases. Noncystic nephrons are destroyed, undergo apoptosis and disappear, leaving behind a kidney displaying extensive replacement by cysts that are held in place by thick bands of fibrotic material and a strikingly reduced amount of functioning parenchyma. From this point forward the polycystic kidney is consumed by most of the same devastating fibrotic processes that push other chronic renal diseases to the end stages.[106,107] A few patients with ADPKD live to normal life expectancies and escape ESRD. Although the basis of this favorable outcome is unknown, it might be a reflection of the polycystin genotype, PKD2 being milder than PKD1, and of other determinants that govern the number of cysts that develop, their intrarenal distribution and the proliferation rates of the cells within them.

Applications to clinical trial design

Left to expand exponentially, cysts engage increasing amounts of normal parenchyma eventually causing irreversible loss of renal function (Figure 7). Exactly when the Stygian line is crossed in ADPKD is not revealed by measurements of GFR, as compensatory hyperfiltration and tubule hypertrophy camouflage the occult demise of functioning parenchyma. The contributions made by cysts to the decline in function lessens when processes involving the secondary extracellular matrix, inflammation and fibrosis become dominant forces of destruction, as is the case in most chronic progressive renal disorders. The age range at which the effects that are dependent on cyst volume and the secondary effects intersect remains to be determined in individual patients.

Therapies that prevent cysts from forming, as demonstrated in the double-null PCK/Brattleboro animals, would be expected to preserve renal function indefinitely.

The effectiveness of therapies administered after most of the cysts are formed depends on the extent to which renal parenchyma has been irreversibly damaged and secondary forces initiated. At fairly early stages of disease, as illustrated by the administration of AVP V2 receptor blockers to cystic mice,[48,49,108] there is a good chance of keeping GFR within a normal range for many years. By contrast, administration of agents fairly late in the course of the disease, after GFR has started to decline, might be much less effective, as demonstrated by clinical trials of mTOR inhibitors during the past 2 years.[109–113]

As GFR does not adequately identify adverse effects of cysts on parenchymal function early in the course of the disease do we have an appropriate surrogate marker? We think we do. Sequential measurements of total kidney and cyst volumes are currently being evaluated as markers of ADPKD progression. The reasoning behind this strategy is that by determining the number and the rate of enlargement of expanding cysts one monitors actual disease progression and encroachment on normal tissue.[18,19,52,54] Studies in young experimental animals show that treating cystic disease before irreversible renal injury occurs reduces the growth of cysts and reduces the development of renal insufficiency.[54] Treatment of patients fairly early in the course of the disease should slow the rate of increase in kidney volume and delay the onset of renal insufficiency. Further in this regard, measurements of total kidney volume by MRI are well tolerated by young children, which permits the evaluation of therapies decades before changes in GFR might be expected to develop.

Matching the therapeutic agent under study to an appropriate end point is also important. Measurements of kidney volume seem best suited for patients with GFR values in stage 1 chronic kidney disease (>90 ml/min/1.73 m^2) who are treated with drugs targeting cell proliferation and/or fluid secretion. Stage 2 chronic kidney disease (60–89 ml/min/1.73 m^2) might be too late for these types of intervention, as a reduction in GFR to levels unquestionably less than normal by current standards of measurement means that a fairly large fraction of the functioning renal mass might have already been destroyed. In patients with stage 2 chronic kidney disease, the fibrosis process is in full force and a further reduction in kidney volume alone might not over-ride the ESRD fibrosis processes, which seem to be driven by a multitude of factors.[106,107] In such cases, anti-inflammatory, antiangiogenic and antifibrotic agents, using GFR as the principal indicator of therapeutic efficacy, might be more appropriate remedies than reducing volume growth.

Conclusions

In conclusion, renal insufficiency in patients with ADPKD is primarily the consequence of cyst formation and expansion. The mass effect of expanding cysts slows and blocks the flow of urine in noncystic tubules and disrupts delicate vascular relationships in the cortex and medulla, which leads to secondary interstitial inflammation and fibrosis, common to most cases of ESRD. Preventing cyst formation and slowing cyst growth fairly early in the disease course delays disease progression in experimental animals and should prolong useful renal function in patients with ADPKD.

Review criteria

A PubMed search was performed on 3 January, 2011 without restriction on publication date using the search terms "polycystic kidney", "ADPKD", "renal cysts", "kidney volume", "cyst volume", "renal biomarkers", "tubulo-interstitial-inflammation", "renal fibrosis", "ureteral obstruction", "renal insufficiency", "renal failure", "chemokine", "cytokine", "growth factors" and "extracellular matrix". The search was restricted to papers published in English. Relevant publications were identified and reviewed. Other supportive information and referenced works are derived from the authors' knowledge of the published literature and personal experience.

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Author contributions

All authors contributed equally to all aspects of this Review.