Polycystic Kidney Disease

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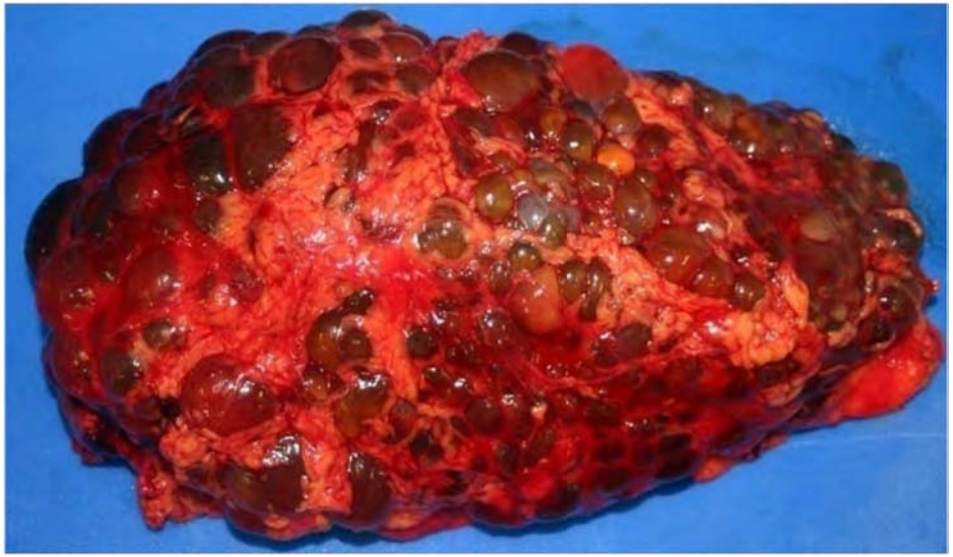
Autosomal Dominant Polycystic Kidney Disease

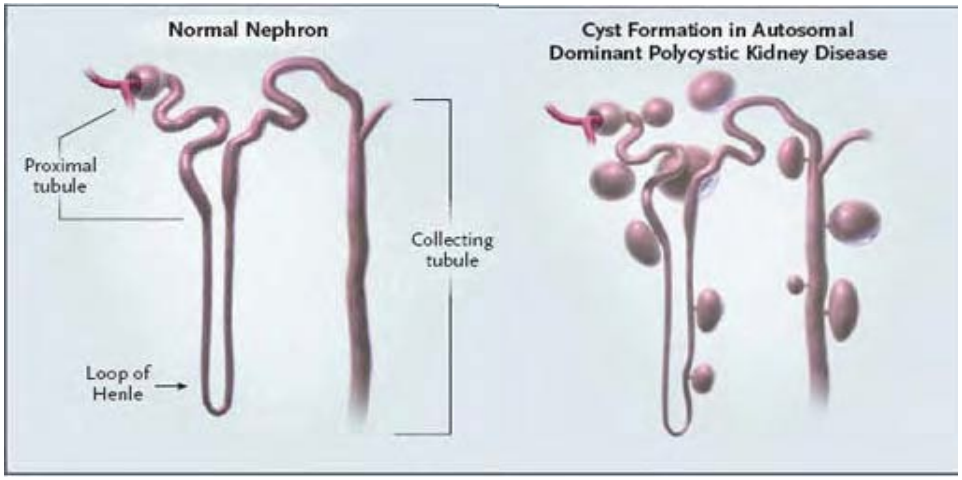
- **Incidence**: 1:1000 live births

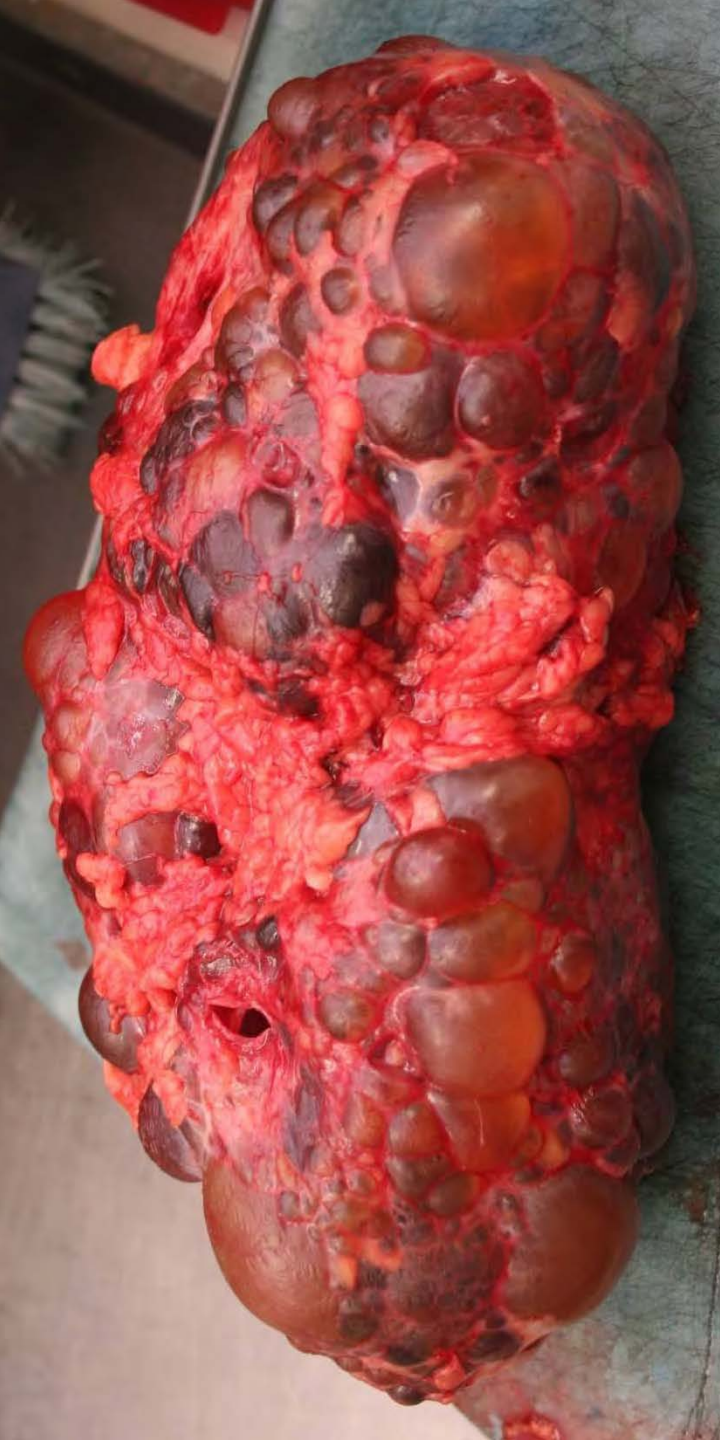
- **Characteristic feature:** multiple fluid-filled cysts in the kidney, liver, pancreas and other organs

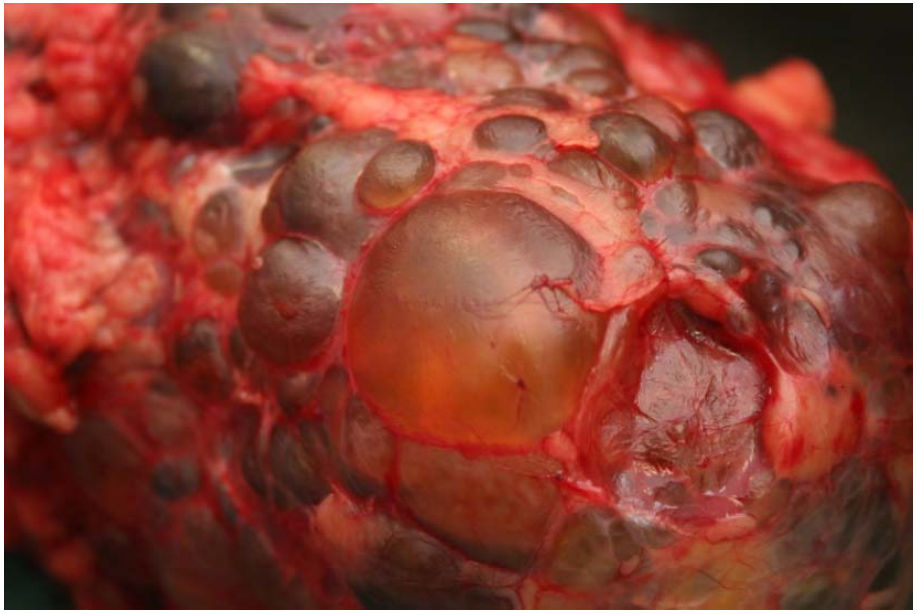
- **Renal manifestations**
  - 50% develop kidney failure by age 60
  - hypertension
  - renal pain
  - haematuria
  - urinary tract infection
  - nephrolithiasis

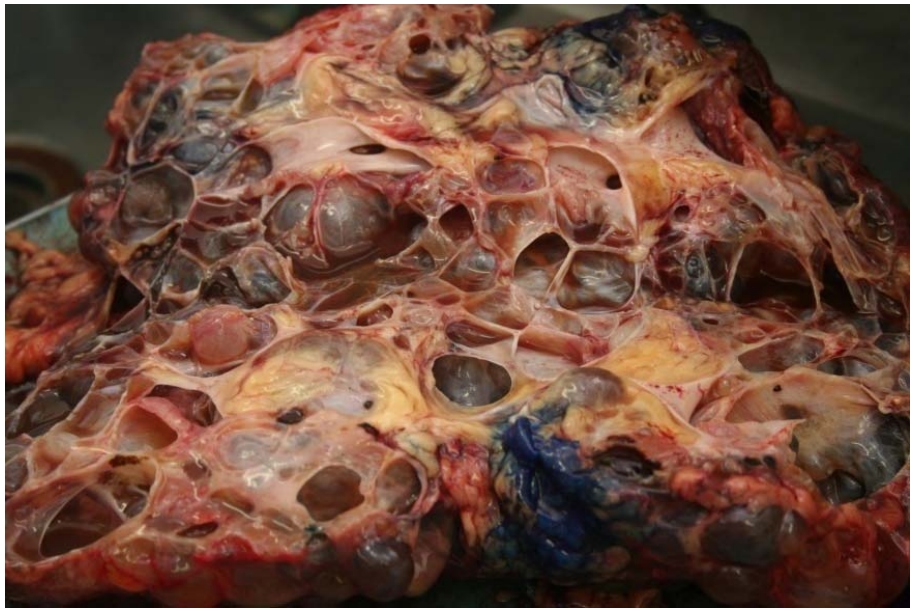
- **Other associations**
  - CVS (valve abnormalities, LVH)
  - GIT (liver/bilary cysts, diverticulum, hernia)
  - Intracranial and other aneurysms
  - Vascular aneurysm
  - Male infertility

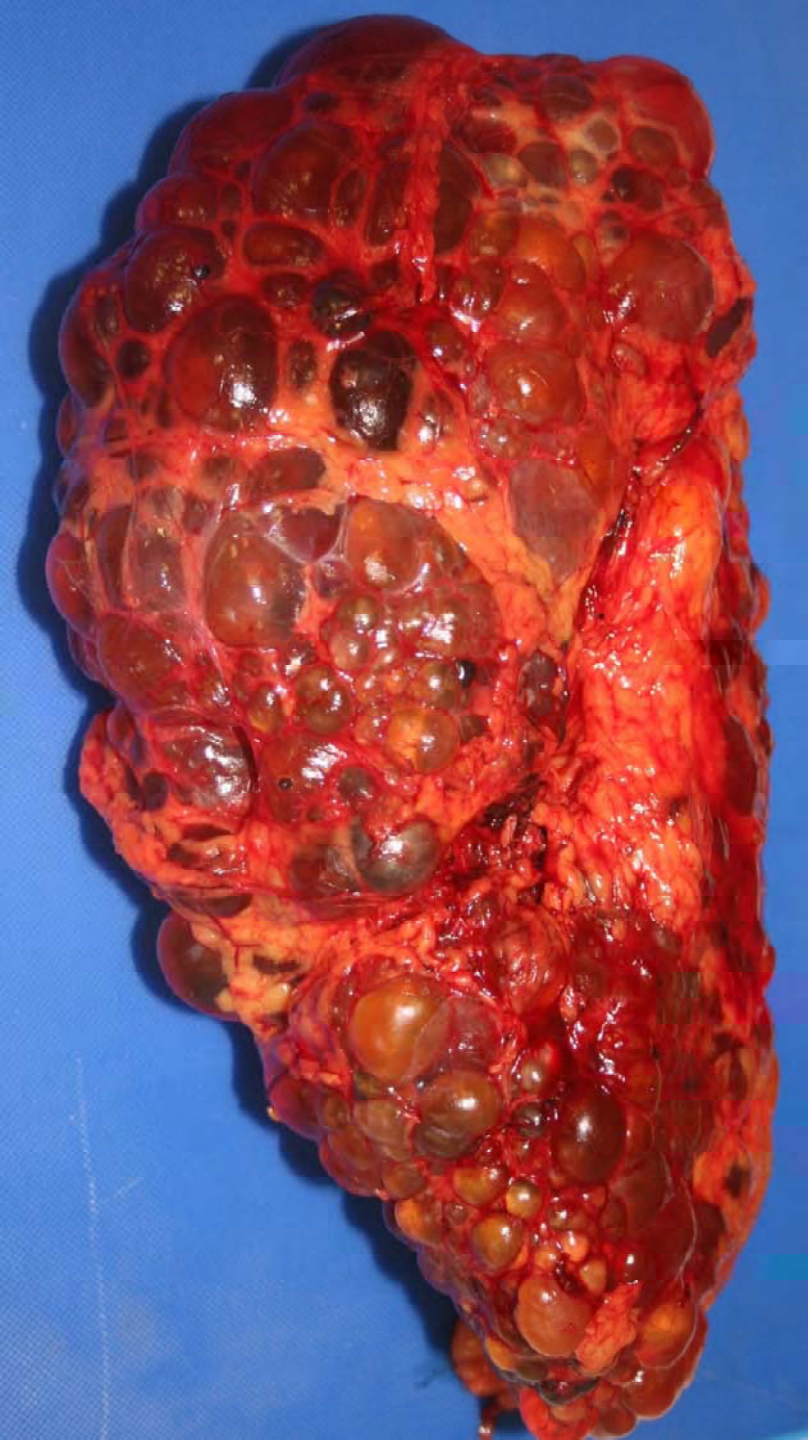


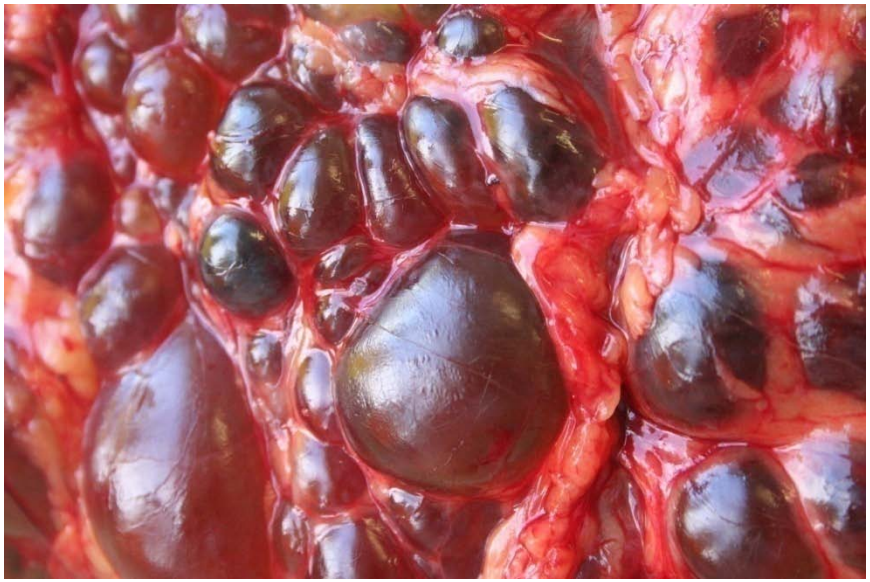


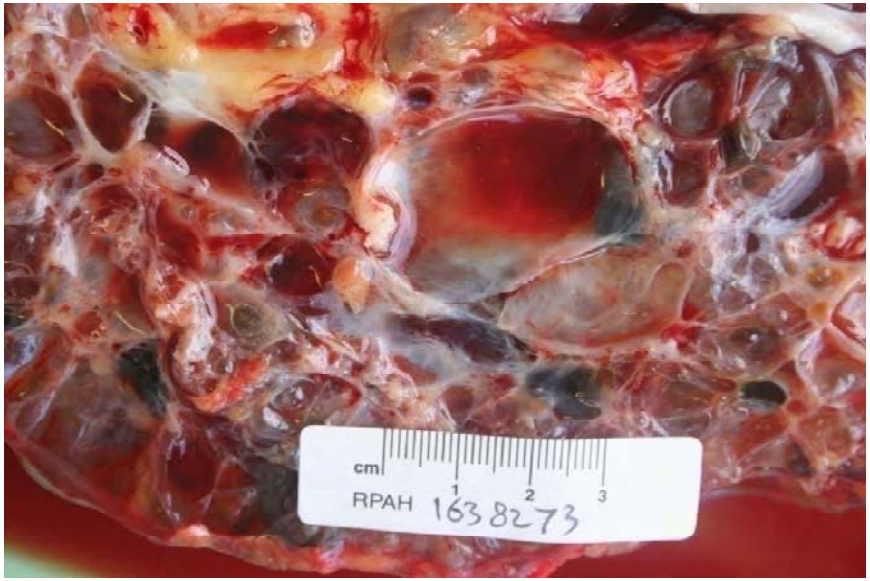











cm
1
2
3
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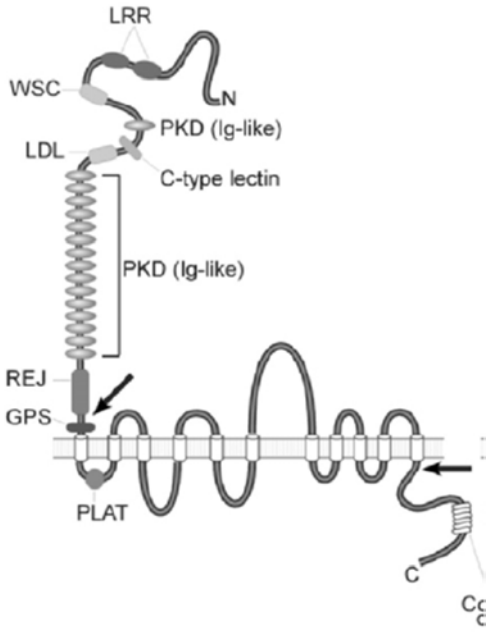
PKD1 gene

- PKD1 gene (16p13.3 ; 53 kB, 46 exons, 15 kB mRNA, encodes polycystin-1 protein)
- Mutations account for 85% of all ADPKD
- 270+ different types of mutations described (produce truncated protein; often unique to a single family; missense mutations much less common)
- Gene complexity makes mutation screening labour-intensive
- Mutation of the 5' portion of the gene *may* have a more severe phenotype

Polycystin-1

- Large transmembrane protein (m.w. 500 kD)
- Large extracellular N-terminal region contains several specific motifs
- 11 transmembrane domains
- Multifunctional protein with important functions in cell/matrix adhesion and ciliary function
- C-terminal tail enters nucleus and regulates cell signaling after cleavage, possibly initiated by polycystin-2 and initiated by mechanical stimuli

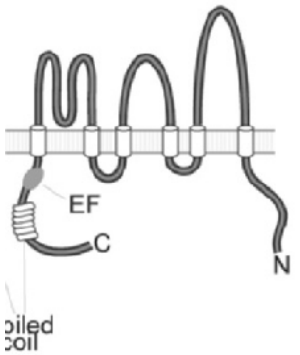


PKD2 gene

- PKD2 gene (4q21 ; 15 exons, 5 kB, mRNA, encodes polycystin-2 protein)
- Mutations account for 15% of all ADPKD
- 70+ different types of mutations (truncated protein, unique to a single family; missense mutations much less common)
- No obvious genotype/phenotype correlations identified
- Disease phenotype of both PKD1 and PKD2 mutations are similar, except that PKD2 mutation is characterised by later onset of end-stage kidney failure

Polycystin-2 (= TRPP2)

- Transmembrane protein (m.w. 150 kD)
- Cytoplasmic N- and C-terminal domains with 6 transmembrane domains
- New member of the transient receptor potential (TRP) family of ion channels, as it is ion channel with some selectivity for calcium ions
- Functions in multiple locations, including plasma membrane, endoplasmic reticulum and the primary cilia
- PC-1 and PC-2 function together as well as independently in a variety of subcellular compartments



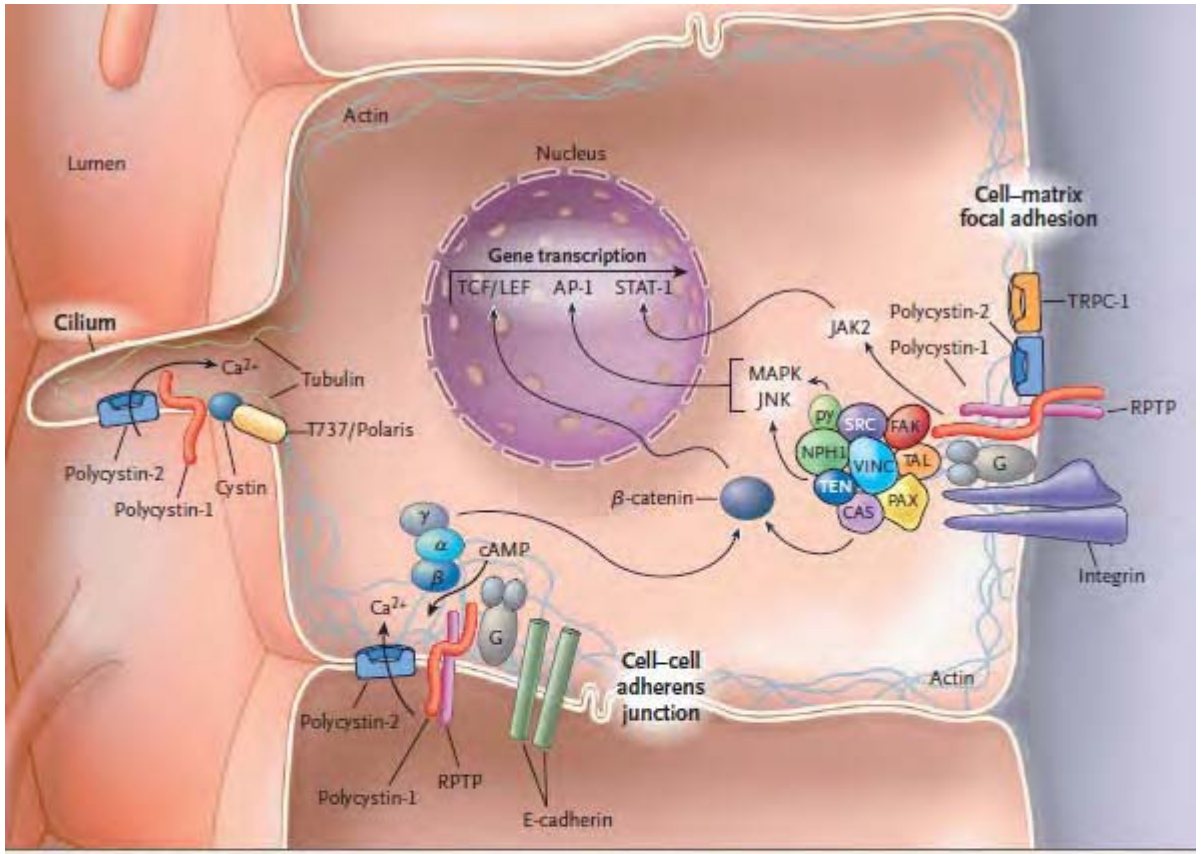


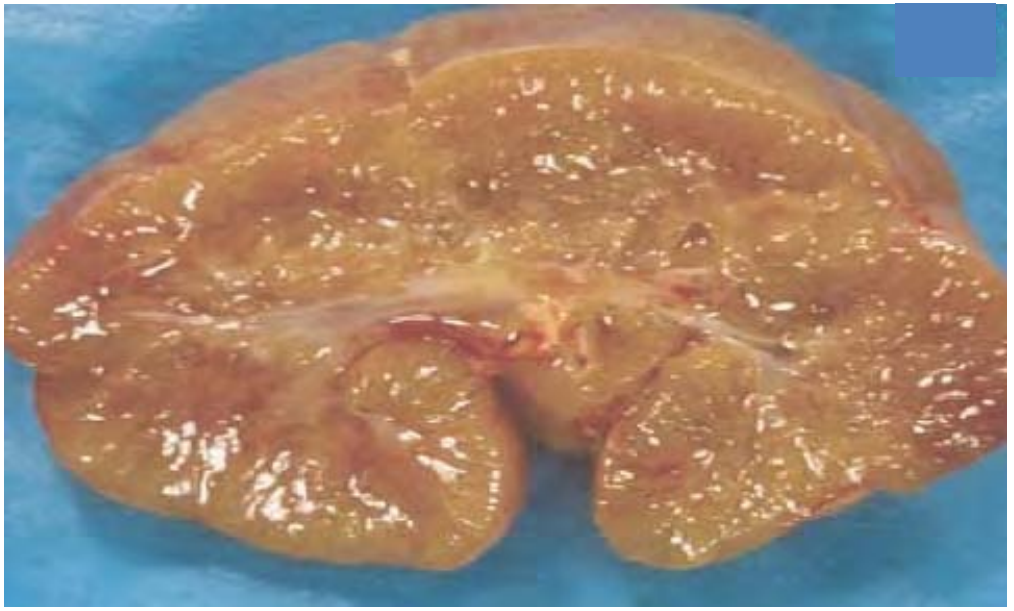
Figure 4. The Function of Polycystin-1.

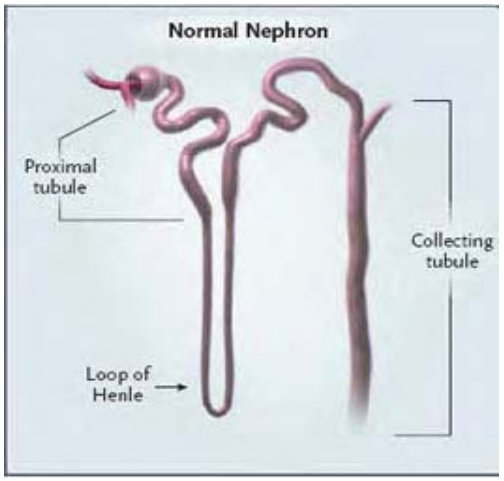
Polycystin-1 complexes are found at the cell–matrix interface, cell–cell contacts, and luminal cilium, where they are thought to function as sensors of the extracellular environment and interact with proteins of the cell membrane and actin and tubulin cytoskeleton and transduce signals by means of intracellular phosphorylation cascades to regulate gene transcription in the nucleus. Polycystin-1 interacts with polycystin-2, $\alpha_2\beta_1$ integrin, receptor protein tyrosine phosphatase (RPTP), and E-cadherin at cell membranes, in focal adhesions, at adherens junctions, and in collecting-duct central cilia. On the intracellular face, polycystin-1 interacts with the focal adhesion proteins talin (TAL), paxillin (PAX), vinculin (VINC), focal adhesion kinase (FAK), c-src (SRC), p130-cas (CAS), nephrocystin (NPH1), the proline-rich kinase pyk-2 (Py), and tensin (TEN) and with the adherens junction proteins β-, α-, and γ-catenin and E-cadherin, which may regulate cell–matrix focal adhesion and cell–cell adhesion, respectively. Polycystin-2 and transient receptor potential calcium-channel 1 (TRPC-1) can facilitate calcium influx, which may act as an intracellular second messenger. The second messenger cyclic AMP (cAMP), as well as G proteins (G), may regulate the function of polycystins through interactions with the polycystin-1 C-terminal at defined sites. The polycystin-1 C-terminal contains sites for phosphorylation on serines (by protein kinases A and X) and on tyrosines (by c-src and focal adhesion kinase), as well as proline-rich src homology 3 (SH_3) and putative WW sites. Signal-transduction cascades induced by the polycystin complex include those of the Wnt pathway (by means of β-catenin and T-cell [TCF] and lymphoid-enhancing [LEF] transcription factors), the focal adhesion pathway (by means of MAP kinase [MAPK], JUN kinase [JNK], and activating protein 1 [AP-1] transcription), and the JAK2–STAT1 pathways, suggesting transcriptional regulation of proliferation, apoptosis, epithelial differentiation, polarity, adhesion, migration, cell shape, and tubular diameter, which are all components of renal morphogenesis.

Wilson PD. N Engl J Med 2004;350:151-64.

Autosomal Recessive Polycystic Kidney Disease

- **Incidence**: 1:20 000 live births
- **Characteristic feature:** childhood disease consisting of cystic kidney with congenital hepatic fibrosis
- **Manifestations**
  - majority present *in utero* or in newborn with kidney enlargement and biliary dysgenesis
  - less commonly present with late-onset portal hypertension or cholangitis
- **Less common AR-inherited cystic kidney diseases**
  - Nephronophthisis (NPHP)
  - Bardet-Biedl syndrome (BBS)
  - Joubert syndrome (JBTS)
  - Meckel-Gruber syndrome (MKS)





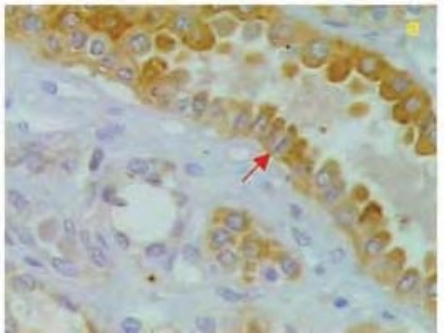
Wilson PD. N Engl J Med 2004;350:151-64; Gattone VH. Curr Opin Pharmacol 2005, 5:1–8

PKHD1 gene

- PKHD1 gene (6p21, 500kb, 67 exons, 16kB mRNA, encodes fibrocystin/polyductin)
- 100% of all ARPKD
- 300+ disease-causing mutations
- Majority of mutations unique to a single family
- Two truncating mutations may be associated with a more severe phenotype

Fibrocystin

- Large receptor-like membrane-associated protein (m.w. 450 kD)
- Single transmembrane domain, large extracellular N-terminal region and small cytoplasmaic C-terminal tail
- Has functional similarity to hepatocyte growth factor receptor, and therefore may function as a receptor or a ligand
- Expressed in cortical and medullary collecting ducts of the kidney as well as biliary and pancreatic ducts (consistent with disease distribution of ARPKD).
- Located in multiple subcellular locations (basolateral membrane, cytoplasm, cilia)
- Cleavage of the ecotodomain and generation of cytoplasmic fragment translocate to the nucleus, possibly in response to stimulation of intracellular calcium or protein kinase C activation. May form a complex with PC-2, but its exact function is unknown.



Menezes et al. Kidney Int, 66 2004, 1345–1355

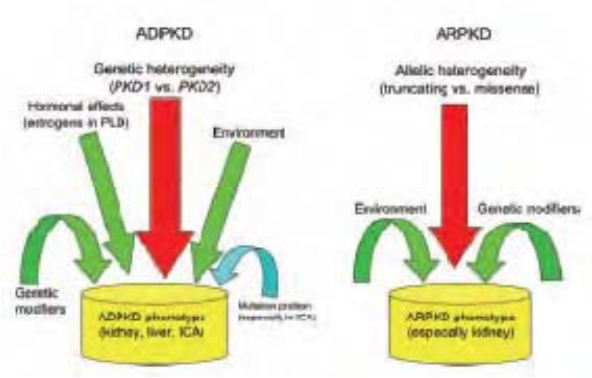
However, there is significant inter- and intra-family **phenotypic heterogeneity in ADPKD.** This, together with the fact that cyst formation is focal (only 1-5% of nephrons develop cysts) and slowly progressive through life, suggest that PKD gene mutation predisposes the kidney to cyst formation, but that other factors, possibly environmental or epigenetic mechanisms, must contribute to cyst formation.

Some theories to explain these observations include:

1. Two-hit hypothesis (loss of heterozygosity, LOH)
   i.e. germ-line mutation in one allele
   + acquired postnatal somatic mutation in the second allele
   (?toxins, ?aging ? Ischaemia, episodes of tubular injury)

2. Haploinsufficiency, modifier genes, epigenetic mechanisms

3. Altered stoichometry of the polycystins



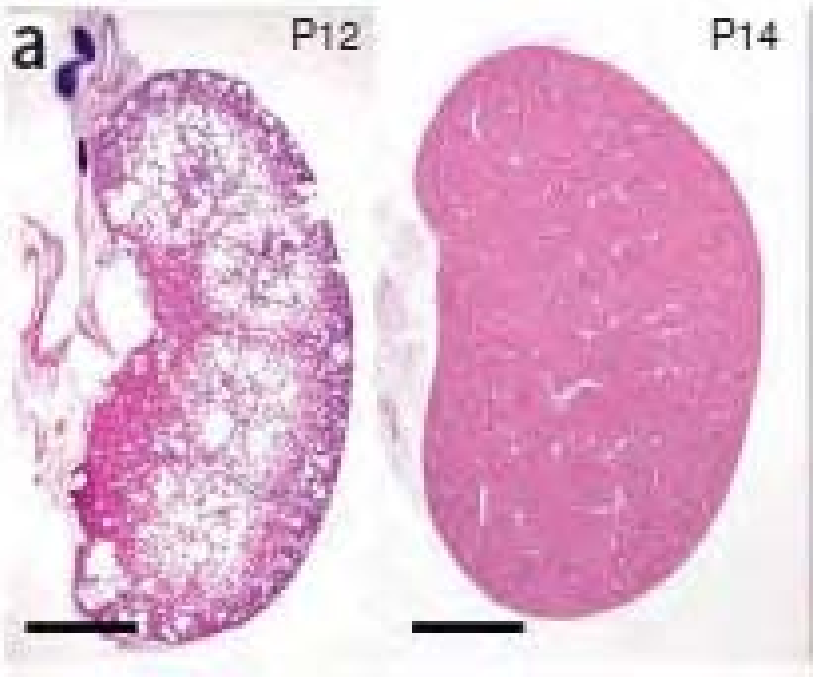
The phenotypic heterogeneity and natural history of ADPKD is also highly relevant to the design of clinical trials and future therapeutics:

1. Identifying who will progress (e.g. renal volume >1500 mls, new biomarkers)

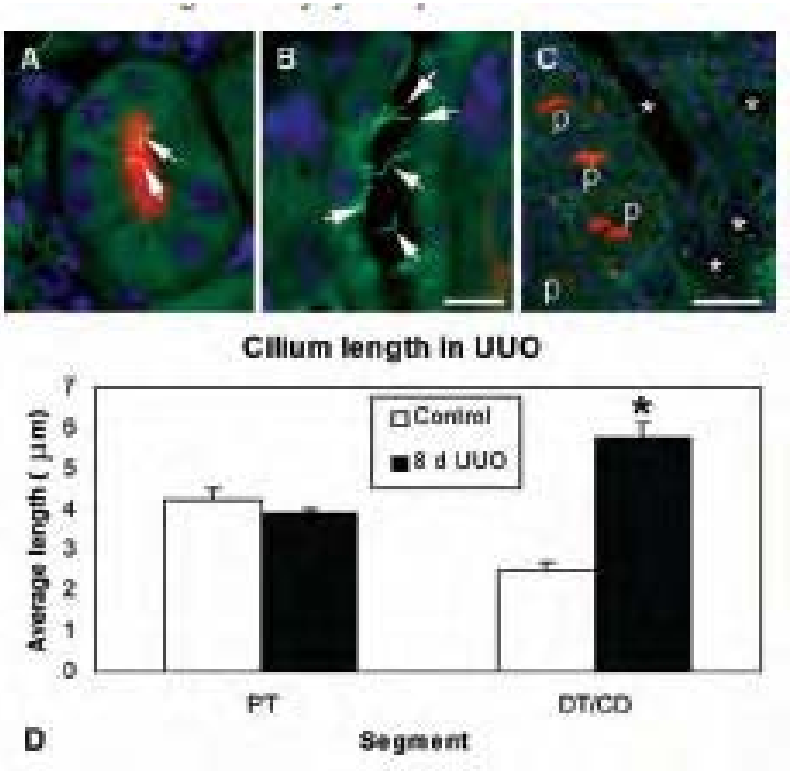
2. Need to treat asymptomatic patients for decades and therefore intervention needs to be well tolerated (e.g. low general toxicity and/or targeted to the site cyst formation)

In ADPKD, is cyst formation partly due to episodes of acute tubular injury later in life?

Polycystin-1 is needed for normal tubulogenesis in kidney development



Polycystins are also probably required for Normal tubular regeneration later in life



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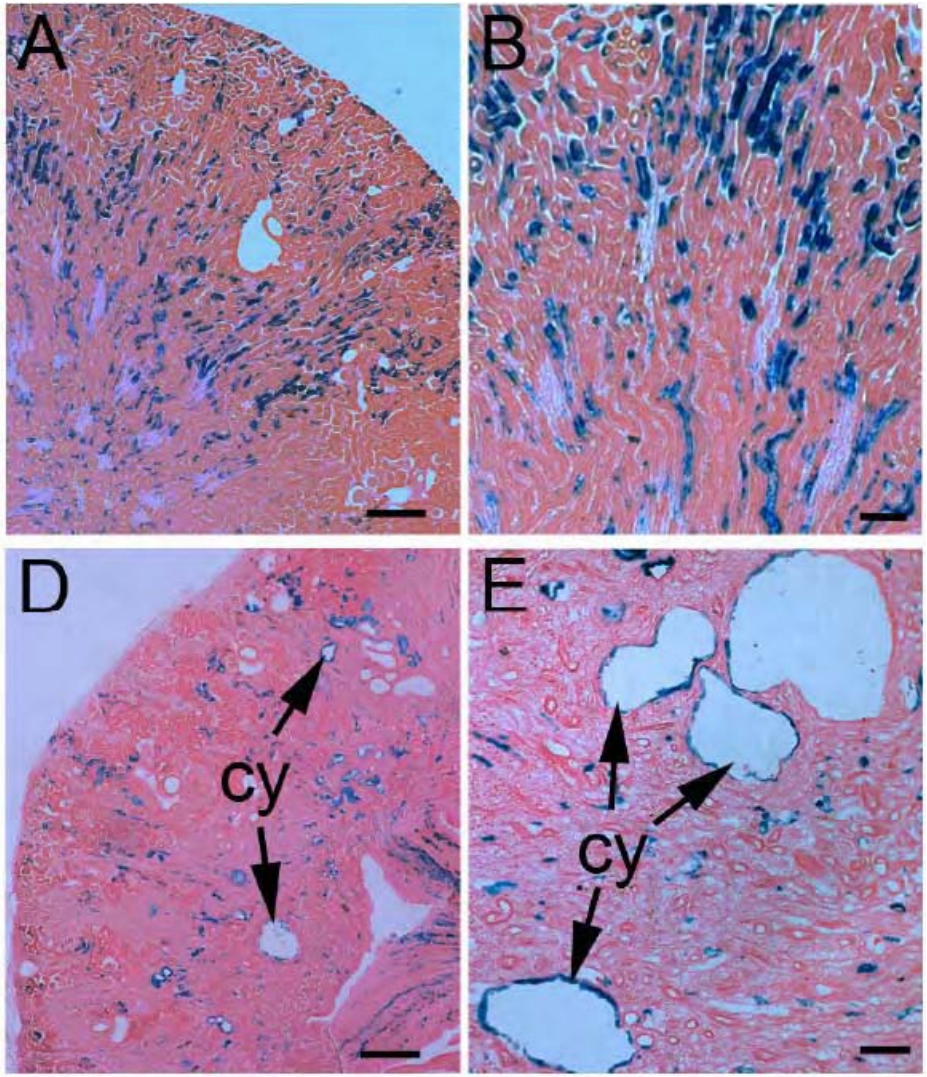
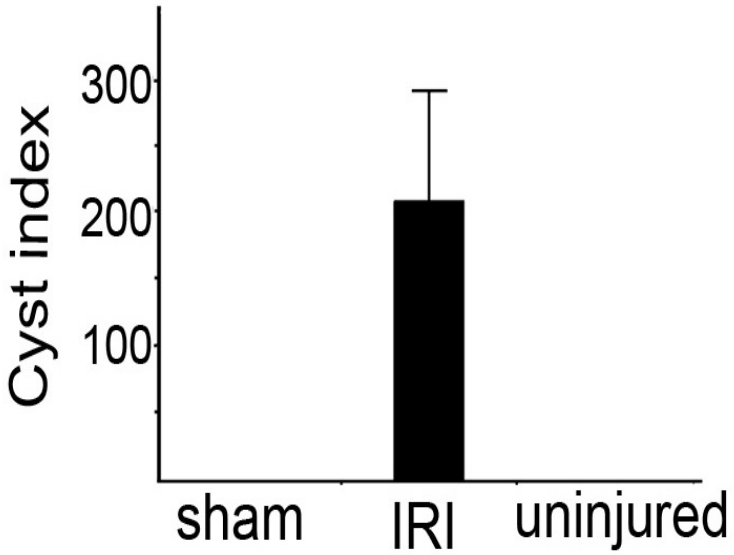


Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia

Vishal Patel[1], Ling Li[2], Patricia Cobo-Stark[1], Xinli Shao[1], Stefan Somlo[3], Fangming Lin[2,4], and Peter Igarashi[1,2,4,*]



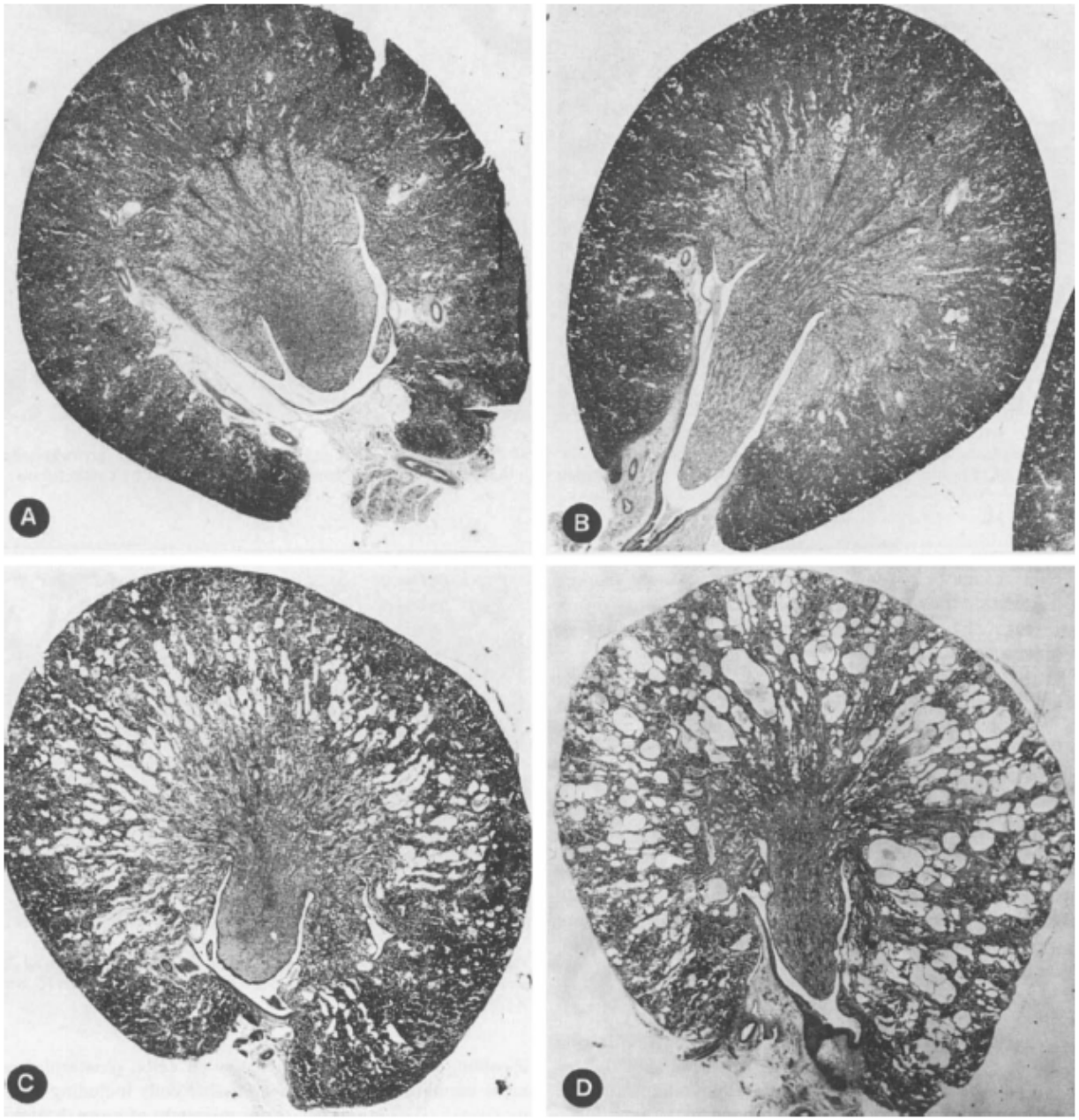
Chemically-induced model of PKD

• Sprague-Dawley rats

• 1.06% diet containing 2-amino-4,5-diphenylthiazole (DPT) for 4, 8, 12, and 30 weeks

•DPT induced two types of tubular change:
- progressive cystic change of all CDs;
-foci of tubular hyperplasia in the cortex, which with time became atrophic.

•These changes have a number of features in common with human ARPKD and m s)



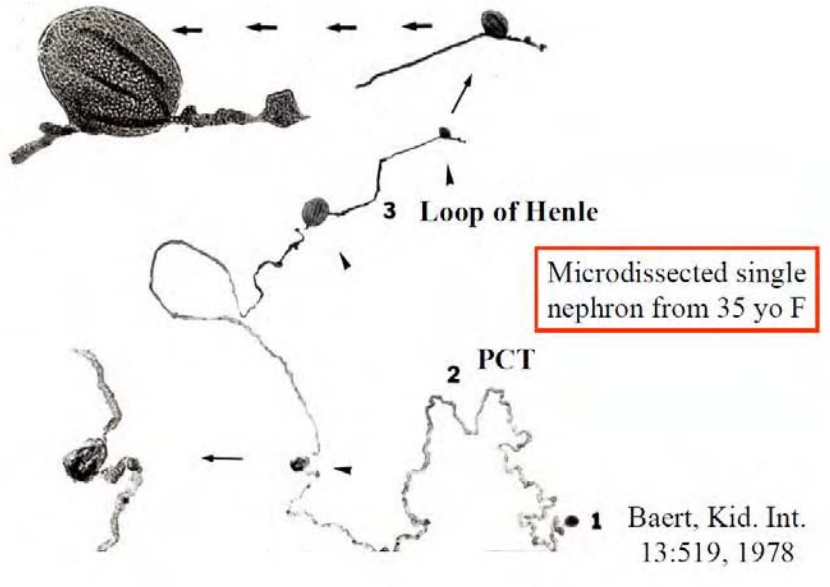
g. 1. *Renal cystic changes after 4* (A), *8* (B), *12* (C), *and 30* (D) *weeks of DPT treatment*. A and B: ×9, C and D: ×6.

Carone et al. Kidney Int 33 1988, 8-13; Darmedy et al. Lancet, 547-550, 1970

Birth and Growth of Cysts in ADPKD

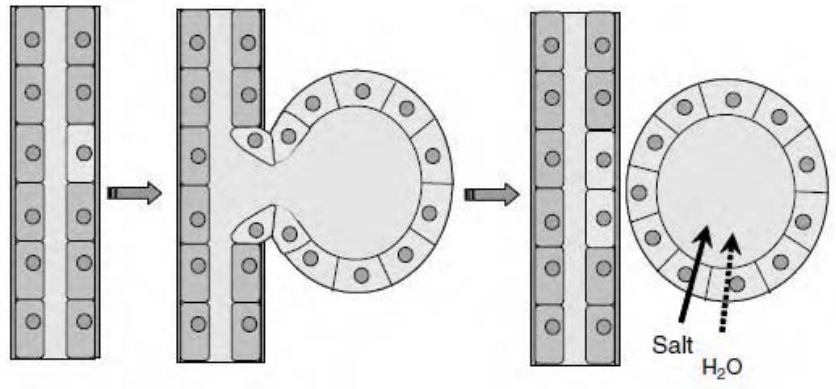
Nature of cyst formation

- Hundreds, ranging in size from a pinhead to the size of a grapefruit
- Cysts arise in renal tubules when epithelial cells undergo focal proliferation
- Initially this leads to “diverticula” from the nephron, which progressively increase in size



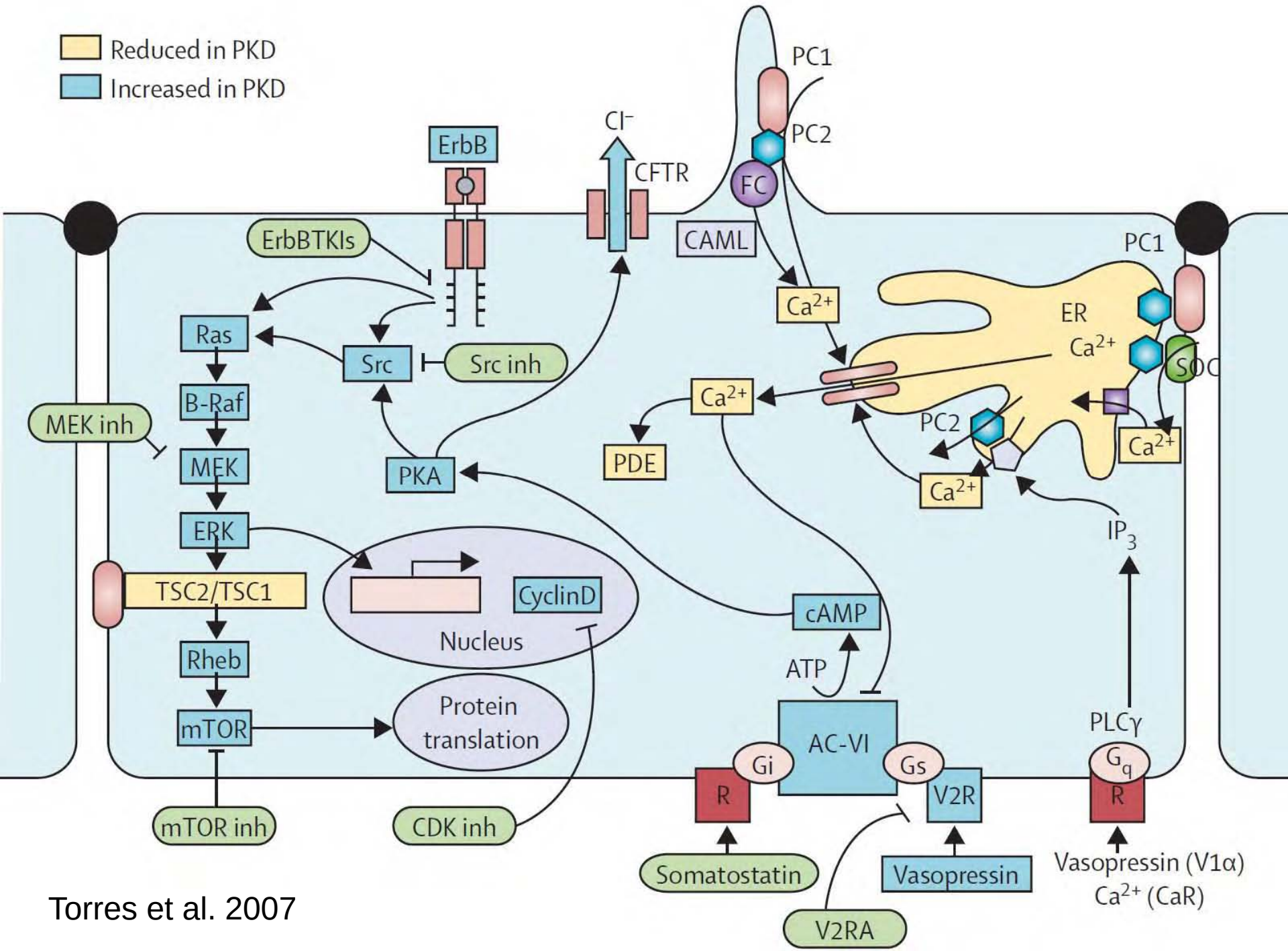
Postulated mechanisms of cyst formation

- An early and sustained proliferation of tubular epithelial cells due to loss of function of growth repressor genes (polycystins, fibrocystin)
- Abnormal solute-driven luminal chloride and fluid secretion, which is largely cAMP and vasopressin – dependent, and a manifestation of epithelial dedifferentiation due to loss of polycystins/fibrocystin



Phenotypic characteristics of normal tubular and cystic epithelial cells

Normal tubular epithelial cell	Cystic tubular epithelial cell
Normal growth - Low rate of cell division - Low rate of apoptosis - Planar polarity **Differentiated** - polarized - reabsorptive - normal concentrating capacity - form branching tubules in collagen gels - normal cell-cell/matrix interactions	**Abnormal growth** - high rate of cell division - high rate of apoptosis - loss of planar polarity **De-Differentiated** - polarity defects (e.g. EGF receptor) - secretory - reduced concentrating capacity - form cysts in collagen gels - abnormal cell-cell/matrix interactions (e.g. reduced E-cadherin) N.B. Phenotype of the cystic epithelial cell is not 'malignant' but it is also not unambiguous. It probably evolves with time and local environmental conditions



Torres et al. 2007

Focal nature of cyst formation in ADPKD and slow adult onset

- Every cell of the nephron and collecting harbors the *PKD1* or *PKD2* germline mutation, but only 1-2% of the nephrons or collecting ducts develop cysts.

- Potential explanations of this observation are:

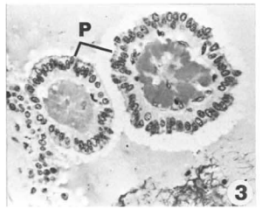
  (i) that ADPKD is due to a “two-hit” mechanism, in which an inherited germline mutation is compounded by a second somatic mutation.

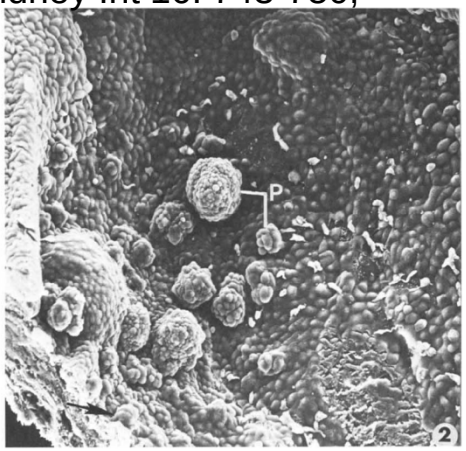
  (ii) Haploinsufficiency

  (iii) Altered stoichometry of the polycystins

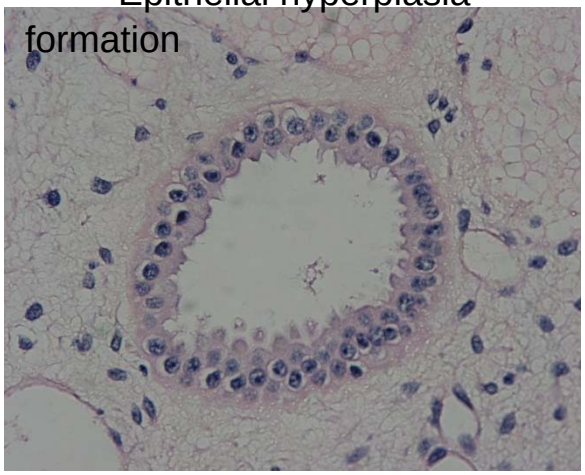
Ultrastructural and histological evidence of cystic epithelial proliferation in human ADPKD

Evan et al. Kidney Int 16: 743-750, 1979

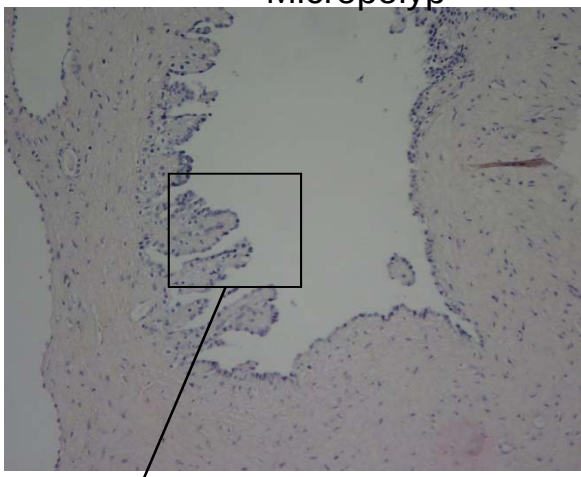


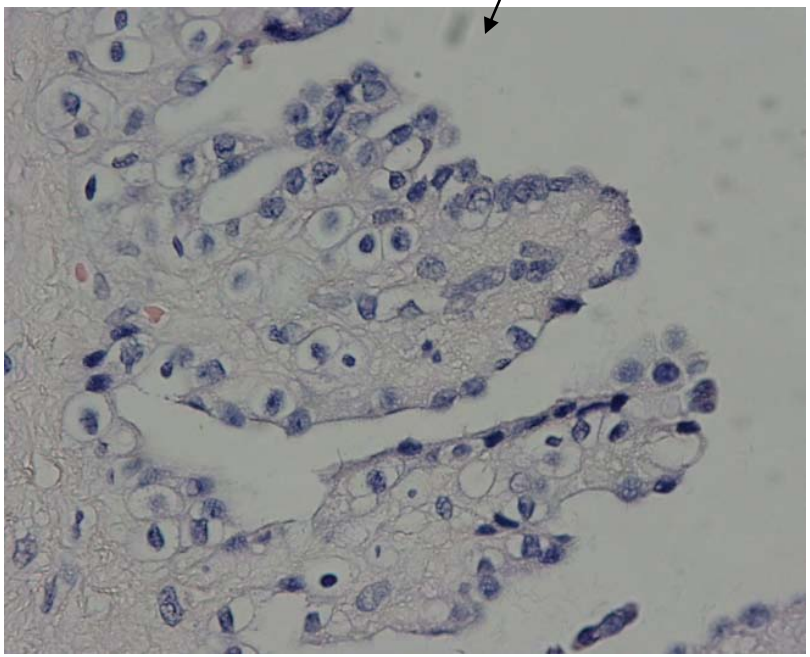


Epithelial hyperplasia formation

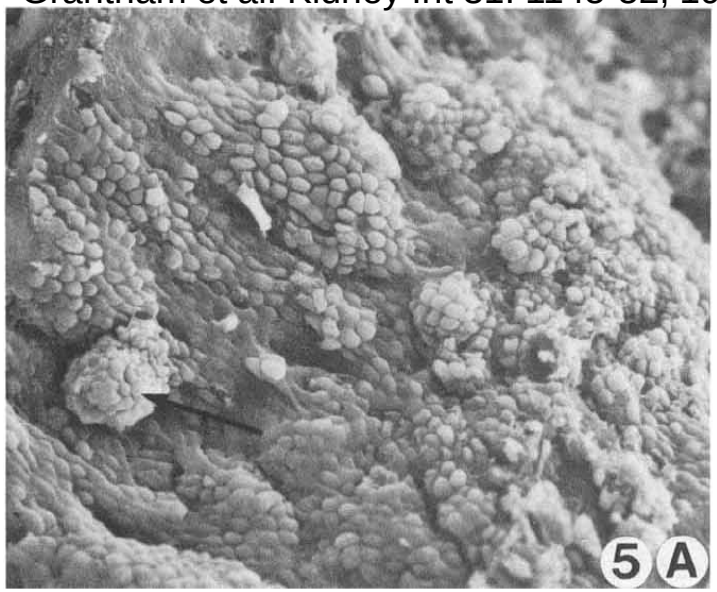


Micropolyp

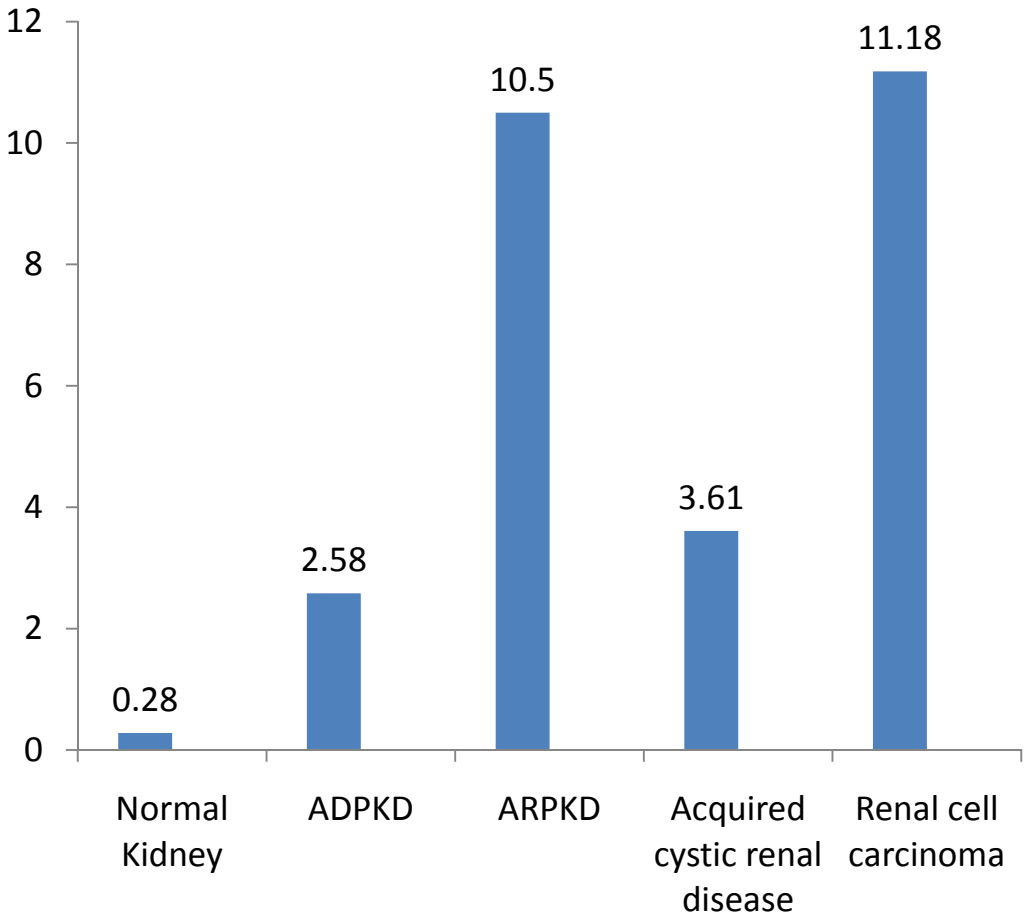


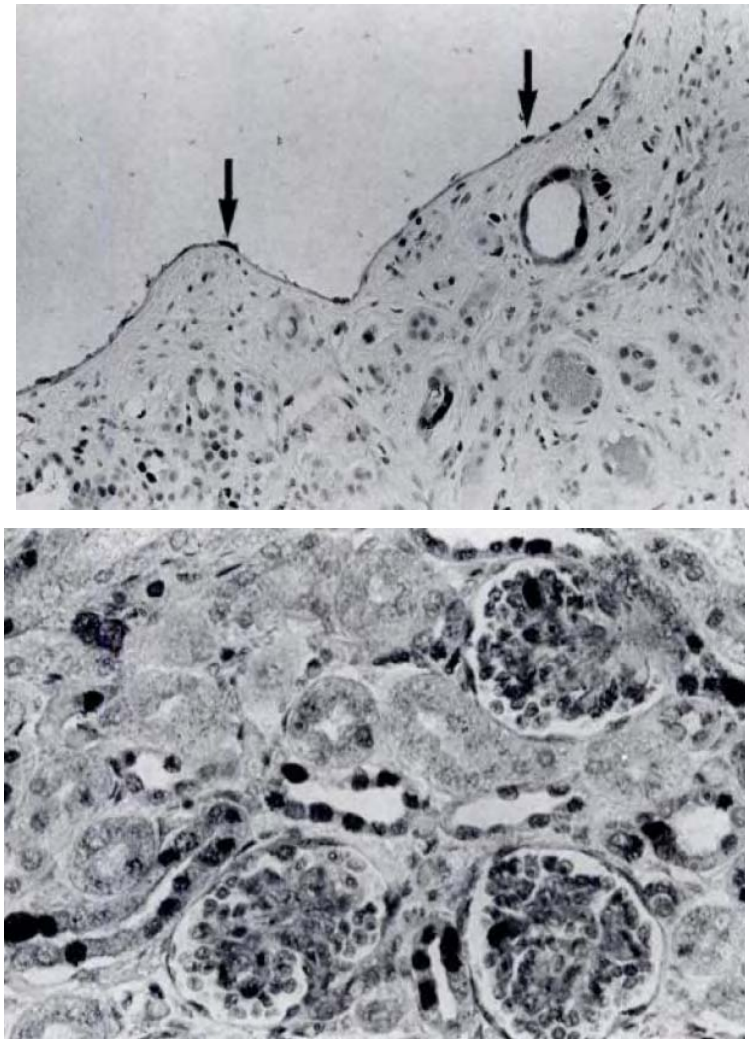


Grantham et al. Kidney Int 31: 1145-52, 1986



Mean proliferative (PCNA) index is increased in human cystic renal diseases





Modified from Nadasdy et al. J Am Soc Nephrol 1995; 5:1462-1468

Which segment of the nephron do cystic epithelial cells arise from?

Table 2. Morphologic characteristics of 387 cysts examined by scanning electron microscopy

Epithelial features	Number of kidneys	Number of cysts	Percent of total cysts
Collecting tubule	7	28	7.2%
Proximal tubule	1	7	1.8%
Glomerular visceral	4	8	2.1%
Not typical of normal tubule segment	10	325	84.0%
Micropolyps and cord–like hyperplasia	6	19	4.9%
Total		387	100.0%

Grantham et al. Kidney Int 31: 1145-52, 1986

ADPKD

Cysts are derived from all nephron segments but both human and PKD1-null mice studies show that they arise predominantly from the distal nephron (LOH, DCT, CD)

- Heggo 0. J Path Bacterlol 1966:91:31 1-3 15.
- Baert L: Kidney Int 1978:13:519-525.
-Faraggiana T et al. Lab Invest 1985:53:575-579
- Verani I,Silva P0. Mod Pathol 1988:1:457-463

ARPKD

Cystic dilatation arises almost exclusively in the collecting duct

- Verani R, Walker P, Silva P0: Pediatr Nephrol 1989:3:37-42
- Heggo 0, Natvig JB. Acta Pathol Microbiol Scand 1965:63:500-512.
- Osathanondh V, Potter EL. Arch Pathol 1964:77:466-473.
- Holth et al. Lab Invest 1990:62:363-369.

Acquired cystic renal disease

Predominantly proximal tubules

- Deck MA, et al. Surg Pathol 1988:1:391-406.
- Ishlkawa I: In: Gardner IW Jr. Bernstein J, Eds. The Cystic Kidney. Boston: Kluwer: 1990:351-377.
-Feiner HD, Katz LA, Gallo OR: Urology 1981:17:260-264.

Morphometric evidence to suggest cyst expansion is due to an increase in cell number and not simply due to 'stretching ' and 'thinning' of individual cells in human ADPKD

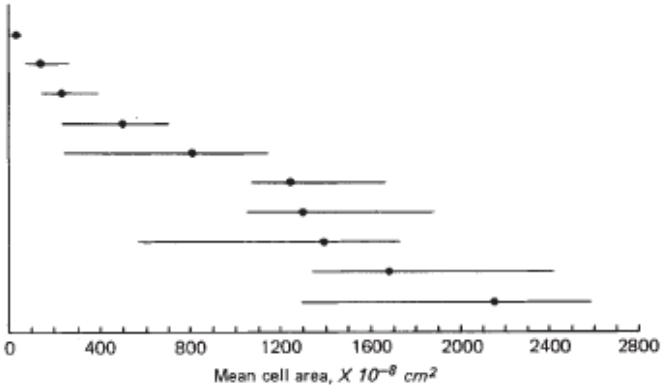


Fig. 7. *Distribution of cell surface areas within 10 cysts.* Range of surface areas shown by horizontal line and mean surface area by the closed circle.

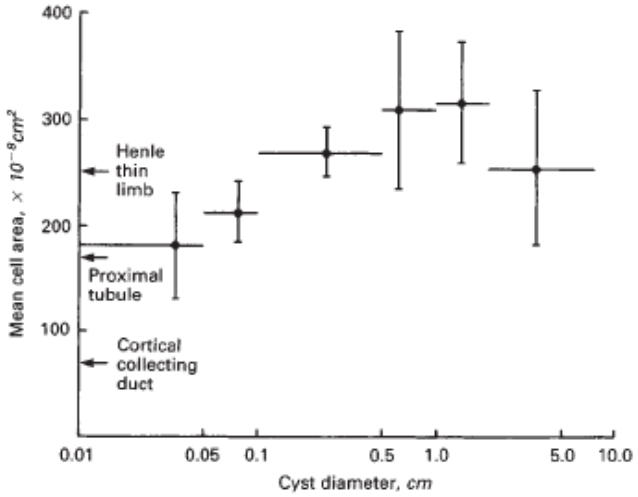


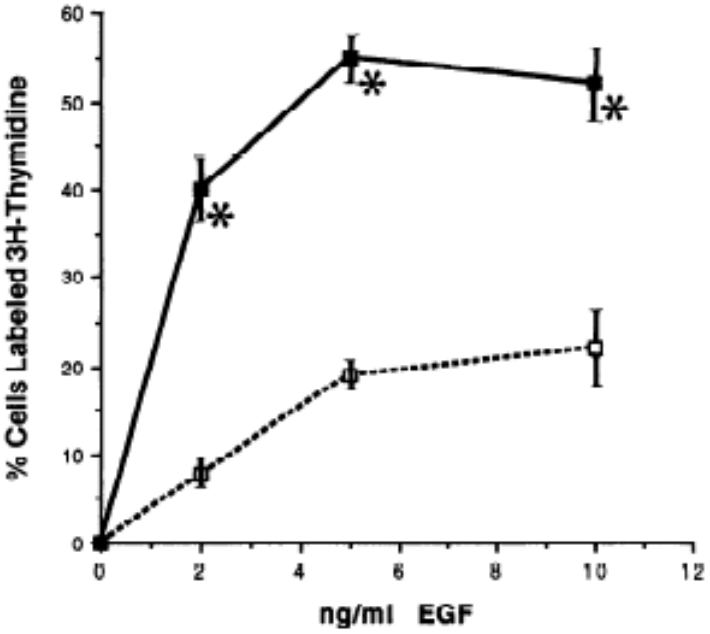
Fig. 8. *Relation between mean cyst diameter and mean cell surface area.* Horizontal lines show range of cyst diameters; vertical lines show ± SEM.

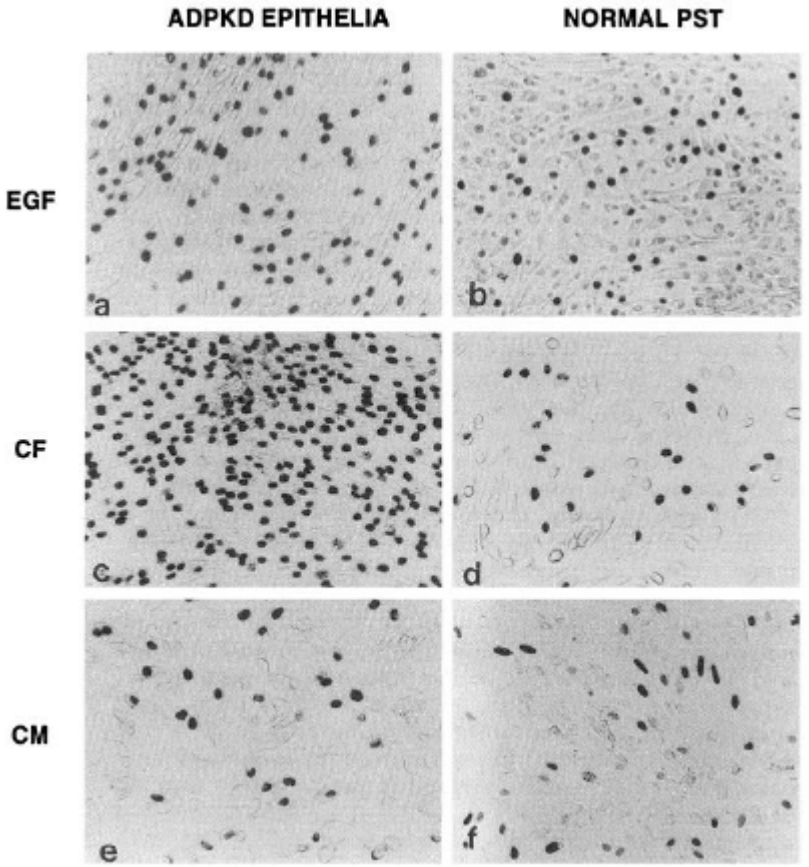
- Normal proximal tubule diameter = 62.4 x 10^{-4} cm
- To grow a 1 mm cyst derived from the proximal tubule to 8 cm, surface area would need to increase from 19.6 x 10^{-4} cm^2 to 201 cm^2, which represents a 100, 000-fold increase.
- However, the surface area of individual cells only increases, at the most 15-fold, from 170 x 10^{-8} to 2530 x 10^{-8} cm^2
- Therefore, cell number must increase at least 10,000-fold to produce an 8 cm cyst

Table 3. Normal values for human renal tubules

	Apical cell[a] width × 10^{-4} cm	Apical cell[b] area × 10^{-8} cm^2	Tubule diameter × 10^{-4} cm^2
Proximal tubule [27]	14.7	170 (1)	62.4 (13)
Henle thin limb [28]	17.9	251 (5)	26.1 (1)
Distal tubule [29]			
pars recta	7.7	47 (2)	76 (1)
macula densa	5.6	24 (2)	
Collecting duct [30]			
dark cells	7.9	49 (1)	
light cells	9.4	70 (29)	

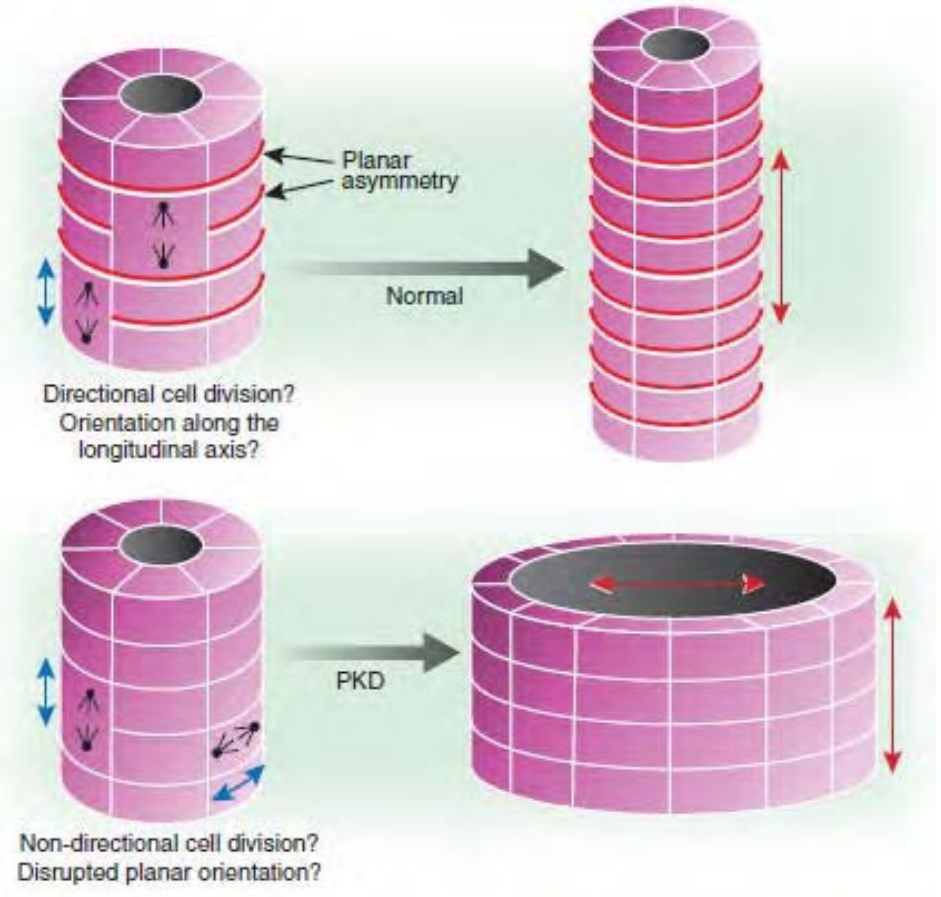
Grantham et al. Kidney Int 31: 1145-52, 1986

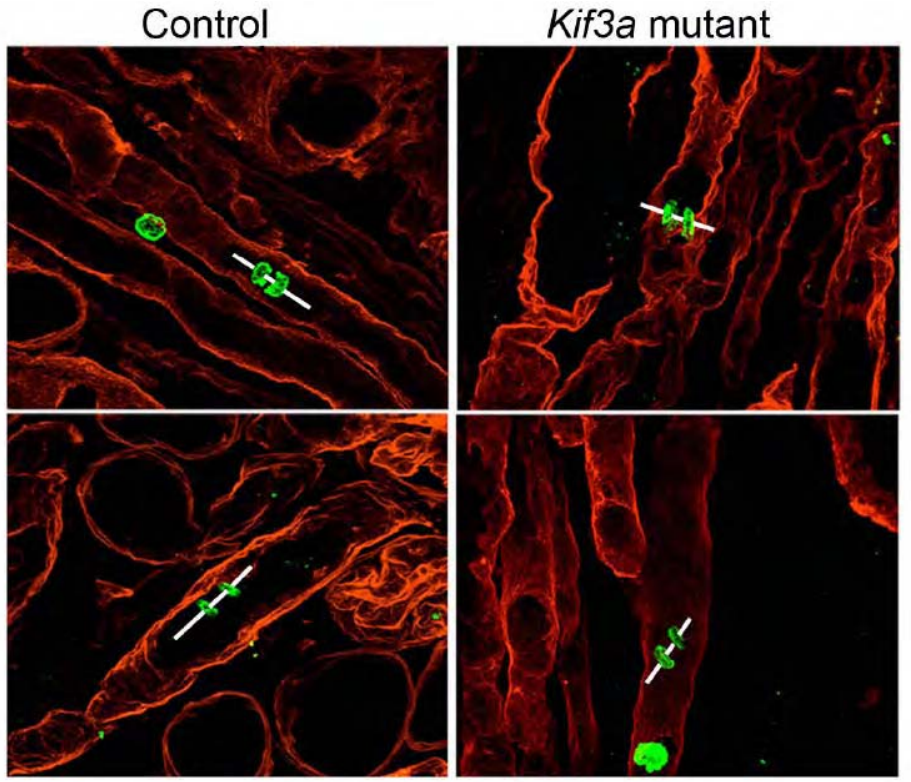




Du and Wilson. Am J Physiol 269, C487-95, 1995

Proliferation is also abnormal in experimental models of polycystic kidney diseases:
Loss of planar cell polarity





Germino 2005

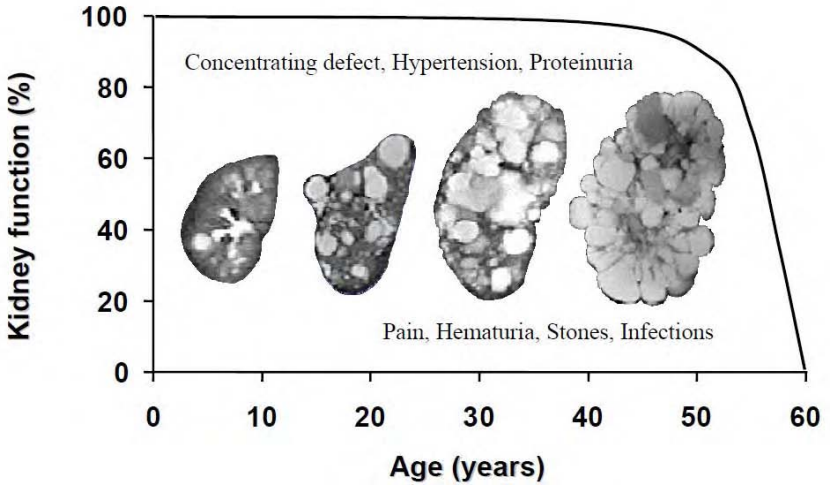
Patel et al. 2008

Natural history of ADPKD and risk factors for progression

In ADPKD, there is a long asymptomatic period when GFR can be well preserved but there is significant kidney enlargement

Risk factors for progression

1. Age at diagnosis (symptomatic) (<30 y.o, renal survival 10 years)
2. Genotype (PKD1 faster than PKD2; Why?)
3. Kidney size (CRISP study; >1500 ml = GFR decreased by -4 ml/min/year)
4. Family history of end-stage renal failure
5. Others: combination of hypertension, diagnosis and gross haematuria before 30, had zero renal survival at age 48



Why does kidney failure occur with cyst expansion?

Not entirely clear, because the cyst burden can be significant, yet GFR is well preserved.

Glomerular haemodynamic factors (that is, increased glomerular hyperfiltration in other types of CKD) do not seem to be have a major role, as:

(i) secondary glomerular injury (FSGS) is not increased in ADPKD

(ii) Uninephrectomy in humans, does not seem to have a detrimental effect on progression to end-stage renal failure (Zeier et al. 1992), whereas in experimental animals it does (Kang et al. 2000, Keier)

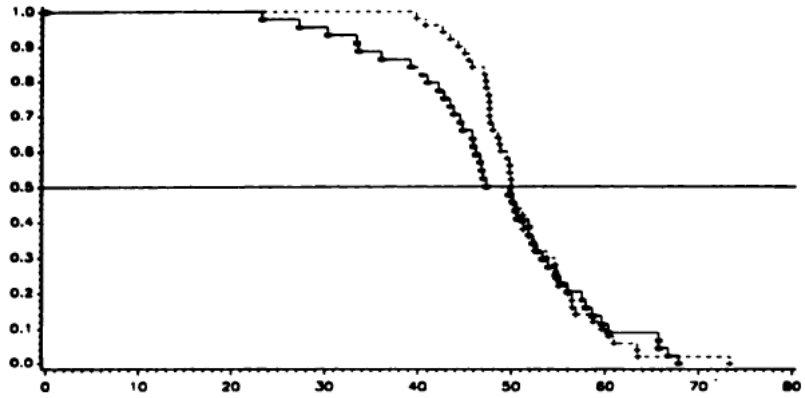


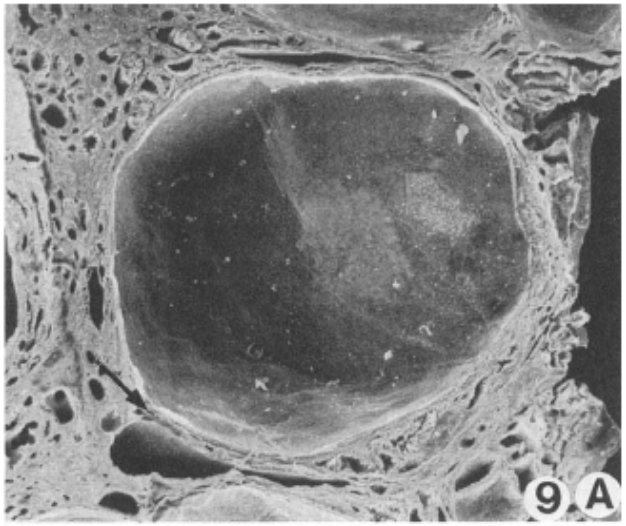
Figure 2. Kaplan-Meier analysis of uninephrectomized versus nonuninephrectomized patients (all patients; *N* = 47). * Uninephrectomized patients; † nonuninephrectomized patients. Panel a, all patients; panel b, male patients; panel c, female patients.

Progression to end-stage renal failure correlates best with the development of interstitial fibrosis and vascular sclerosis

Therefore, the presence of cysts could lead to interstitial fibrosis by:

1. Compression of surrounding tissue with slow enlargement of cysts, leading to loss of peritubular capillary blood supply and tubular obstruction

2. Increased production of profibrotic growth factors and chemokines by cystic epithelial cells



Renal histology in early and advanced human ADPKD

Glomerular pathology

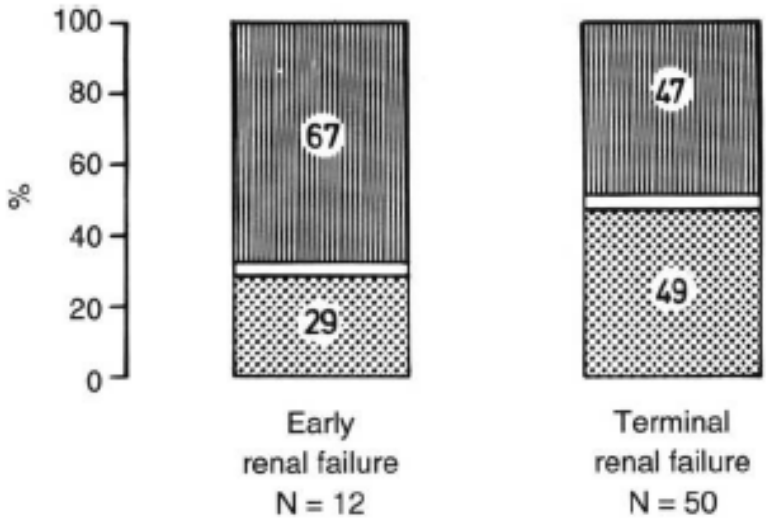
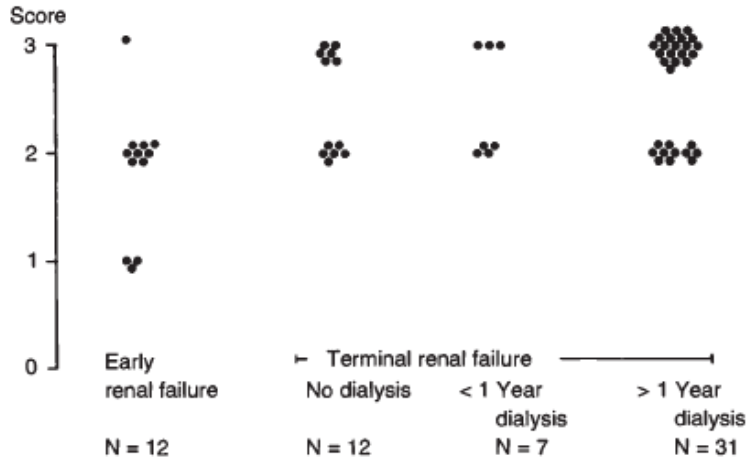
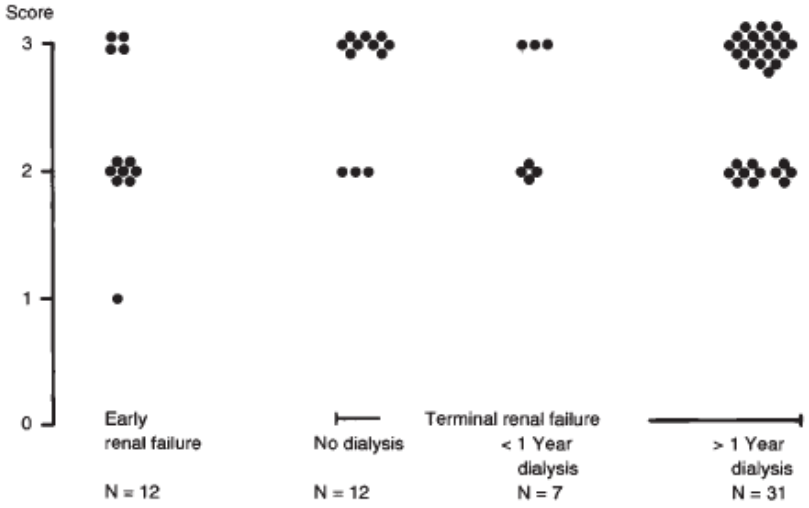


Fig. 3. *Glomerular pathology in patients with ADPKD in early and terminal renal failure.* Symbols are percent glomeruli with: segmental sclerosis (□); (▨) normal glomeruli; or global sclerosis (▩).

Vascular sclerosis

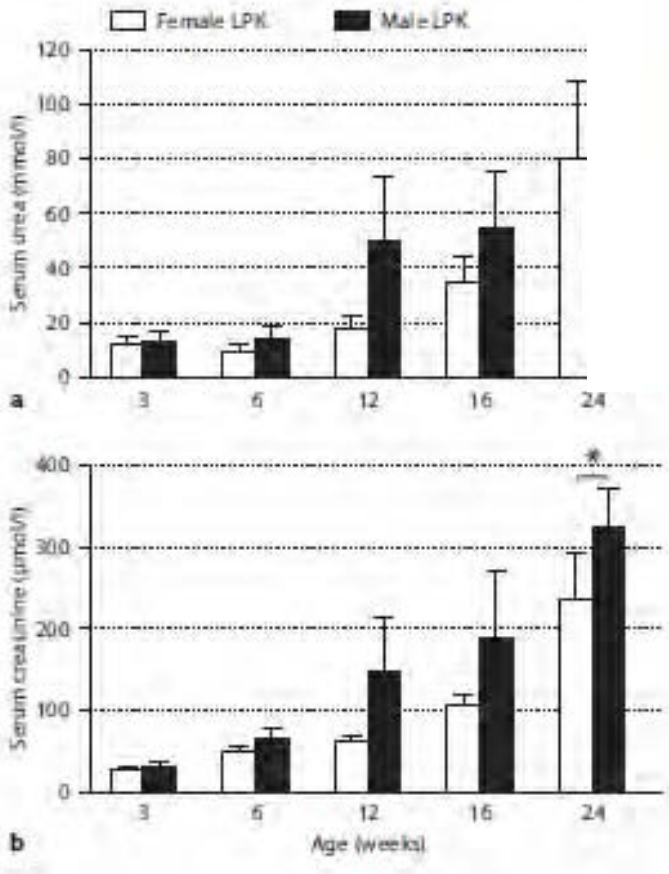


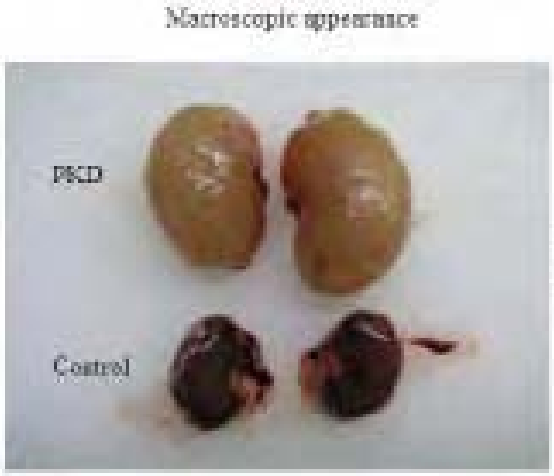
Interstitial fibrosis



Zeier et al. Kidney Int 42, 1259-65,

Onset of interstitial fibrosis coincides with the decline in renal function





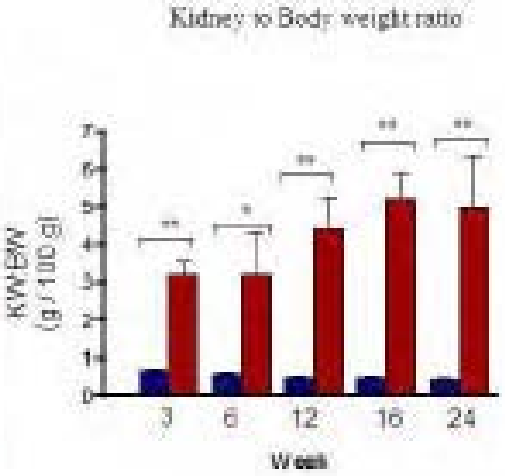


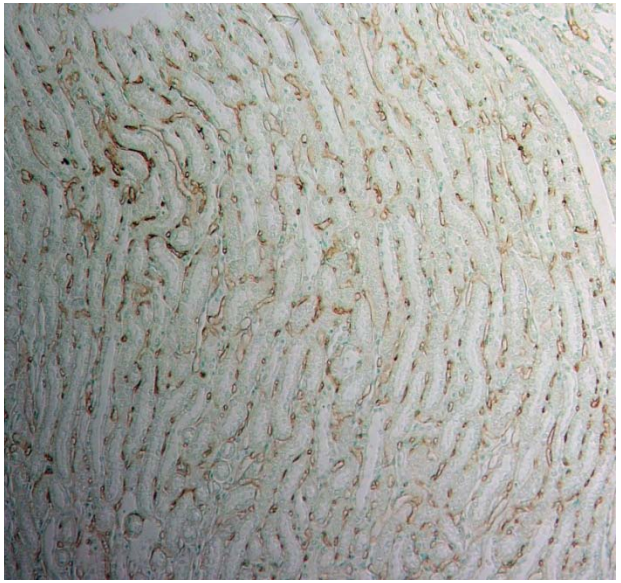
Table 5. Quantification of renal histopathological parameters in age-matched Lewis and LPK rats

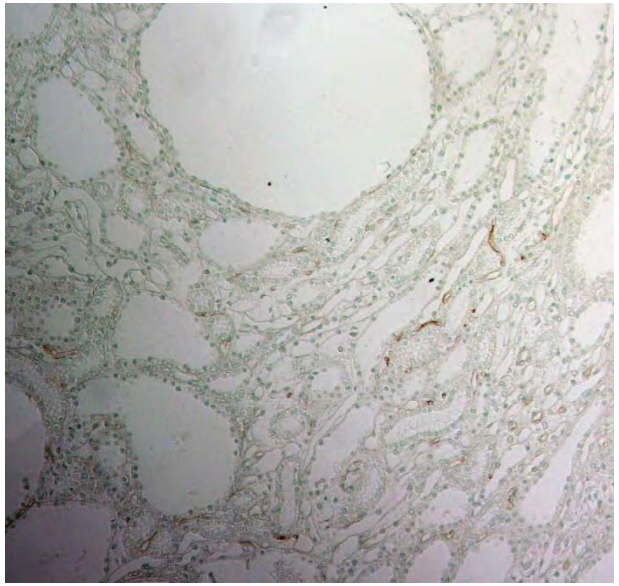
Parameter	1 week		3 weeks		6 weeks		12 weeks		24 weeks		Adjusted R^2 value
	[6]LEW	[6]LPK	[6]LEW	[6]LPK	[6]LEW	[6]LPK	[7]LEW	[7]LPK	[6]LEW	[10]LPK	
Vimentin, cor[a]	0.3 ± 0.2	1.4 ± 0.5	0.1 ± 0.1	3.0 ± 0.4	0.2 ± 0.2	3.3 ± 0.8	0.2 ± 0.2	3.9 ± 0.1	0.2 ± 0.2	4.0 ± 0.1	0.97***
ED-1/mm^2[b]	9.3 ± 0.9	14.0 ± 5.6	5.1 ± 1.1	14.0 ± 2.6	6.0 ± 1.4	34.1 ± 20.3	6.7 ± 1.4	32.6 ± 22.7	8.8 ± 6.8	20.9 ± 13.8	0.44***
Collagen, cx[a]	0.0 ± 0.1	0.2 ± 0.2	0.0 ± 0	0.03 ± 0.1	0.1 ± 0.1	0.23 ± 0.2	0.26 ± 0.2	1.3 ± 0.4	0.4 ± 0.2	2.5 ± 0.5	0.93*
Collagen, med[a]	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0	0.1 ± 0.1	0.6 ± 0.3	0.1 ± 0.1	1.8 ± 0.5	0.89***
α-SMA[c]	5.0 ± 1.2	3.6 ± 1.1	1.1 ± 0.3	3.6 ± 2.4	1.2 ± 0.6	9.7 ± 2.9	1.1 ± 0.6	10.0 ± 3.5	0.8 ± 0.3	12.2 ± 2.3	0.82**

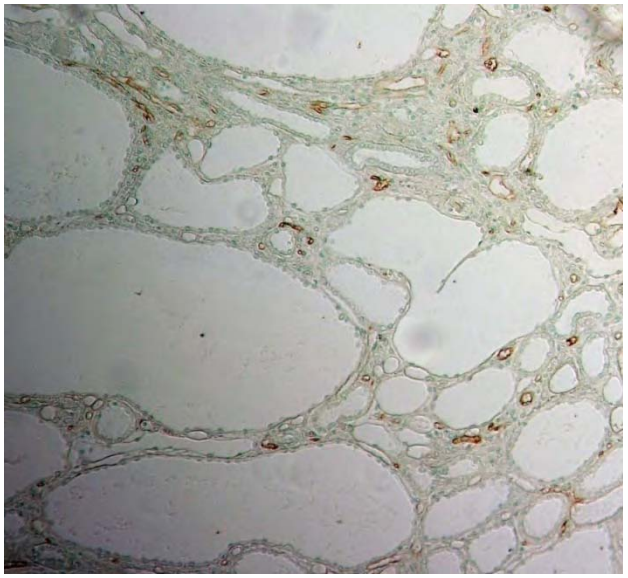
Data represent mean ± SD of combined male and female data.
[a] Tubular vimentin staining and collagen deposition was scored with arbitrary units for the cortex (cx) or medulla (med).
[b] Immunoreactivity for ED-1 was measured as number of immunoreactive cells in midcortical fields per mm^2.
[c] α-Smooth muscle actin (α-SMA) measured as % of cortical area occupied by positive staining for α-SMA.
Minimum number of animals in each group indicated by superscript associated with age/strain column. Significance of strain effect: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

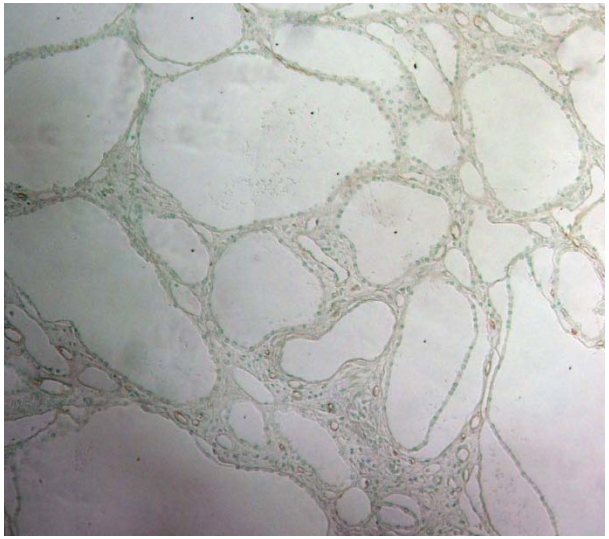
Phillips et al. 2007

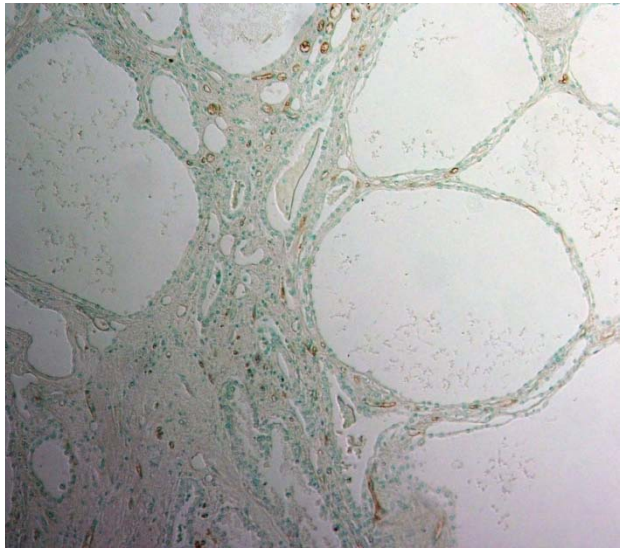
Peritubular capillaries of the outer medulla disappear early in LPK rats











Timecourse of peritubular capillary distribution in the outer medulla of control and LPK rats. Control Lewis rat demonstrates an extensive peritubular capillary network that is more dense than the cortical network. By week 3 LPK rats have very few normal tubules in the outer medulla and the peritubular capillary network is almost non-existent. Some individual capillaries remain. LPK rats at week 6, 12 and 24 demonstrate almost no peritubular capillaries. Some vessels remain and these may be remnant capillaries, vasa recta or new vessels, and these are fewer in weeks 12 and 24 compared to week

O'Brien et al. (unpublished)

Small animal models of PKD

• No currently available genetically- and non-genetically orthologous animal model completely recapitulates human ADPKD or ARPKD

• Homozygous deletion of PKD1 gene is embryonic lethal, whereas heterozygous deletion has focal cyst formation and very slow to progress with little, if any, deterioation in renal function

• The best models for testing new therapies are those with diffuse cyst formation and relatively slow progression

- e.g.
- bpk mouse and jck mouse
- Pck rat
- Han:sprd rat

• Often necessary to prove drug efficacy in more than one model because of these limitations (e.g. one that is genetically and non-genetically orthologous) – jck mouse + pkd^{ws25} mouse

Table 1. *Murine models of polycystic kidney disease*

Model	Transmission	Gene	Protein	Human PKD Phenocopy†*	Left-Right Axis Defect	Cilia Expression‡
			Mouse			
cpk	AR	*Cys1*	Cystin	ARPKD	No	Yes
bpk	AR	*Bicc1*	Bicaudal C	ARPKD	No	Yes§
jcpk	AD/AR	*Bicc1*	Bicaudal C	ADPKD	No	Yes§
orpk	AR	*TgN737Rpw*	Polaris	ARPKD	Yes†	Yes
inv	AR	*Invs*	Inversin	ARPKD	Yes	Yes
jck	AR	*Nek8*	Nek8	ADPKD	No	NE
kat	AR	*Nek1*	Nek1	ADPKD	No	NE
pcy	AR	NI	NI	ADPKD	No	NI
			Rat			
Han:SPRD-cy	AD/AR	NI	NI	ADPKD	No	NI
wpk	AR	NI	NI	ARPKD	No	NI
pck	AR	*Pkhd1*	Fibrocystin	ARPKD	No	NE

Table 2. *Targeted mutations in mouse Pkd1*

Strain/(Ref. No.)	Mutation	Allele*	*Pkd1*$^{-/-}$	Visceral Organ Cysts	Cardiovascular Defects	Edema	Skeletal Defects	*Pkd1*$^{+/-}$
Pkd1^{del34} (62)	Exon 34 deletion	*Pkd1*^{tm1Jsh}	EL	Kidney, pancreas	NE	+	+	Kidney, liver, pancreas cysts
Pkd1^{null} (63)	Exon 4 disruption	*Pkd1*^{tm2Jsh}	EL	Kidney, pancreas	NE	+	+	Kidney, liver, pancreas cysts
Pkd1^{L} (64)	Exon 43–45 deletion	*Pkd1*^{tm1Mau}	EL	Kidney, pancreas	Vascular leak	+	No data	No data
Pkd1$^{del17-21pgeo}$ (65)	Exon 17–21 deletion; IRES lacZ-neo fusion	*Pkd1*^{tm1Rea}	EL	Kidney	Conotruncal defects	+	+	Kidney, liver cysts
Pkd1^{-} (66)	Exon 2–4 deletion with in-frame lacZ	*Pkd1*^{tm1Skh}	EL	Kidney, pancreas	No data	No data	No data	No data
Pkd1^{-} (67)	Exon 2–6 deletion	NA	EL	Kidney	Conotruncal defects	+	No data	No data
Pkd1^{-} (72)	Exon 1 disruption	NA	EL	Kidney, pancreas	NE	+	No data	Kidney, liver cysts
Pkd1^{-} (68)	Point change due to ENU mutagenesis	*Pkd1*^{m1Bei}	EL	Kidney	No data	No data	No data	Kidney, liver, pancreas cysts

Table 3. *Targeted mutations in mouse Pkd2*

Strain/(Ref. No.)	Mutation	Allele*	*Pkd2*$^{-/-}$	Left-Right Axis	Visceral Organ Cysts	Cardiovascular Defects	Edema	Skeletal Defects
Pkd2^{-} (69)	Exon 1 disruption	*Pkd1*^{tm1Som}	EL	No data	Kidney, pancreas	+	+	No data
Pkd2^{WS25} (69)	Exon 1 duplication generating unstable allele	*Pkd1*^{tm2Som}	Viable	No data	Kidney, liver, pancreas	NE	NE	No data
Pkd2^{-LacZ} (70)	Exon 1 deletion *LacZ* "promoter trap"	NA	EL	Randomization; right pulmonary isomerism	Kidney, pancreas	+	+	No data

Guay-Woodford L. Am J Physiol Physiol (2003) 285: F1034–F1049.

Cellular Mechanisms of Cystogenesis and Progression of Cyst Growth

1. Tubular epithelial cell proliferation

2. Tubular epithelial cell dedifferentiation
   - EGF receptor mislocalization
   - fluid secretion
   - reduced cell-cell adhesion
   - increased cell matrix adhesion
   - planar cell polarity

Factors initiating and accelerating tubular epithelial cell proliferation

Genetic Factors

Cilia-localised proteins (polycystin-1, polycystin-2)

Modifier genes

?Post DNA methylation

Circulating Factors

ADH (water intake)

Local Paracrine Factors and Cyst Fluid Constituents

Growth factors (EGF, VEGF, KGF)

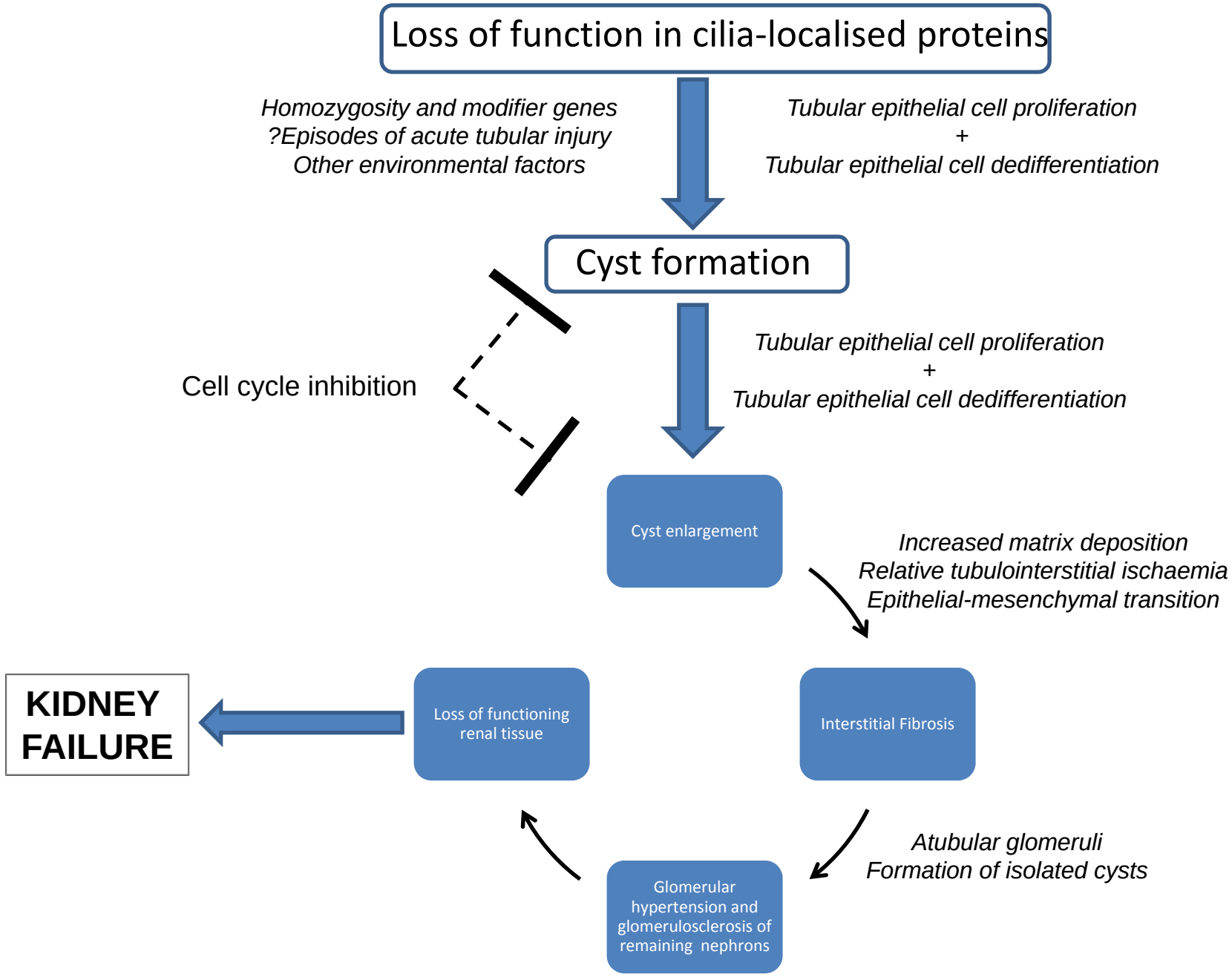
Extracellular matrix (laminin, SPARC)

Angiogenesis

Environmental Factors

Episodes of renal tubular injury

Caffeine


Loss of function in cilia-localised proteins
Homozygosity and modifier genes
?Episodes of acute tubular injury
Other environmental factors
Tubular epithelial cell proliferation
+
Tubular epithelial cell dedifferentiation
Cyst formation
Cell cycle inhibition
Tubular epithelial cell proliferation
+
Tubular epithelial cell dedifferentiation
Cyst enlargement
Increased matrix deposition
Relative tubulointerstitial ischaemia
Epithelial-mesenchymal transition
Interstitial Fibrosis
Atubular glomeruli
Formation of isolated cysts
Glomerular hypertension and glomerulosclerosis of remaining nephrons
Loss of functioning renal tissue
KIDNEY FAILURE

Current approaches to treating polycystic kidney disease

1. Treat hypertension (Target BP 130/80; ACEI/ARB)
2. Maintain appropriate body weight
3. Diet (limit sodium, avoid high-protein diet; limit caffeine intake; adequate water intake)
4. Monitoring disease progression (dependent on renal function)
5. Screening other family members with ultrasound

Future approaches to treat cystic tubule cell proliferation and fluid section

1. Suppressing intracellular cAMP levels (vasopressin receptor antagnoist, somatostatin agonists)

2. Reducing basolateral and luminal chloride transport (NKCCl and CFTR inhibitors)

3. Reducing intracellular calcium (triptolide, calcium channel blockers)

4. Inhibition of cell cycle pathways
   - EGF receptor antagonists
   - Reducing activation of mTOR protein kinase (sirolimus)
   - Reducing cyclin-dependent kinase activation (roscovitine)
   - MEK inhibitors
   - Src inhibitors

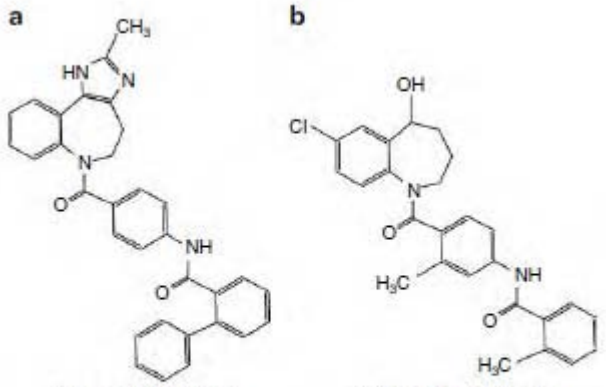
5. Better methods to predict who will progress to end-stage renal failure
   - biomarkers of progression (urinary MCP-1 levels)
   - improvements in genetic testing, epigenetic factors

Vasopressin receptor antagonists

Table 1 | Vasopressin receptor location and functions

Receptor	Localization	Functions
V1a	Vascular smooth muscle	Vasoconstriction, myocardial hypertrophy
	Platelets	Platelet aggregation
	Hepatocytes	Glycogenolysis
	Myometrium	Uterine contraction
V1b[a]	Anterior pituitary	ACTH release
V2	Basolateral membrane collecting tubule	Insertion of AQP2 water channels into apical membrane, induction of AQP2 synthesis
	Vascular endothelium	vWF and factor 8 release
	Vascular smooth muscle	Vasodilatation

ACTH, adrenocorticotropin hormone; AQP2, aquaporin-2.
*Termed V3 in some classification schemes.



Conivaptan (YM-087) Tolvaptan (OPC-41061)

Figure 1 | Structure of the orally active VRAs. (**a**) Conivaptan, a combined V1a/V2 antagonist. (**b**) Tolvaptan, a selective V2 antagonist.

Table 2 | Non-peptide vasopressin antagonists currently under commercial development

Compound	Receptor	Route	Manufacturer
Conivaptan (YM-087)	V1a+V2	i.v.	Astellas (Tokyo, Japan)
Lixivaptan (VPA-985)	V2	Oral	CardioKine (Philadelphia, PA, USA)
Tolvaptan (OPC-41061)	V2	Oral	Otsuka (Tokyo, Japan)
SR-121463	V2	Oral	Sanofi-Aventis (Paris, France)

i.v., intravenous.

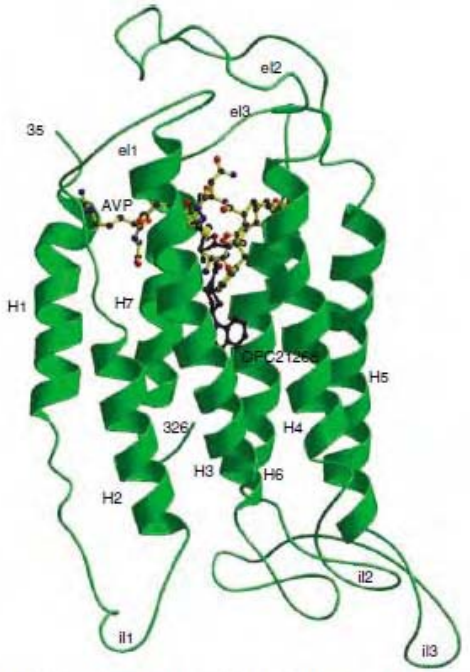


Figure 2 | V2 receptor shown as a ribbon diagram. For the receptor (green), loops labeled 'e' are extracellular and loops labeled 'i' are intracellular. Models of arginine vasopressin (AVP, multicolored) and a V2 receptor antagonist (OPC21268, dark blue) are shown at the sites where they would dock were the other not present. Binding sites are distinct with partial overlap. The antagonist, which lodges deeper in the receptor than AVP, prevents AVP docking but does not interact with the receptor's active site in the H1 helix. The figure was graciously provided by Dr Menachem Shoham and modified with permission from Macion-Dazard *et al.*[8]

Greenberg and Verbalis 12:2124-30, Kidney Int,

Intracellular signal transduction abnormalities

Reduced intracellular calcium

Increased intracellular cAMP

Signal transduction activation (ERK, MEK, B-Raf, mTOR)

Apoptotic regulatory proteins (caspase)

Cell cycle regulatory proteins

Protooncogenes

MicroRNAs

Ultrasound Criteria for Diagnosis of PKD1 in At-Risk Individuals

Positive and negative predictive values 97-100%

Ravine et al, Lancet 343:824, 1994

- Age < 30: at least 2 cysts (unilateral or bilateral)
- Age 30-59: at least 2 cysts/kidney
- Age ≥ 60: at least 4 cysts/kidney

- For PKD2 age 30-59, use 4 or more cysts in both kidneys for sensitivity of 96%

Pei et al, JASN 14:107A, 2003

Manifestations of ADPKD: Kidney

- Cysts throughout both kidneys
- Painful, enlarged kidneys
- Hypertension
- Hematuria
- Cyst infection; pyelonephritis
- Nephrolithiasis
- Impaired concentrating ability
- Quality of life issues
- Progressive loss of kidney function

Kidney Pain in ADPKD

- Diffuse abdominal or unilateral/bilateral flank pain affects up to 60% of adults and 35% of children
- Etiologies of *acute* pain include hemorrhage, cyst infection or pyelonephritis, kidney stones, or growth of cysts
- *Chronic* pain due to massively enlarged kidneys may result from traction on the kidney pedicle, distention of the kidney capsule, or compression of surrounding structures
- The occurrence of pain correlates with kidney size in both adults and children

Hypertension in ADPKD

- 66% of men; 41% of women
- 59% prior to significant loss of GFR; 100% in ESRD
- Associated with LVH
- Correlates with greater kidney and cyst volumes in adults and children

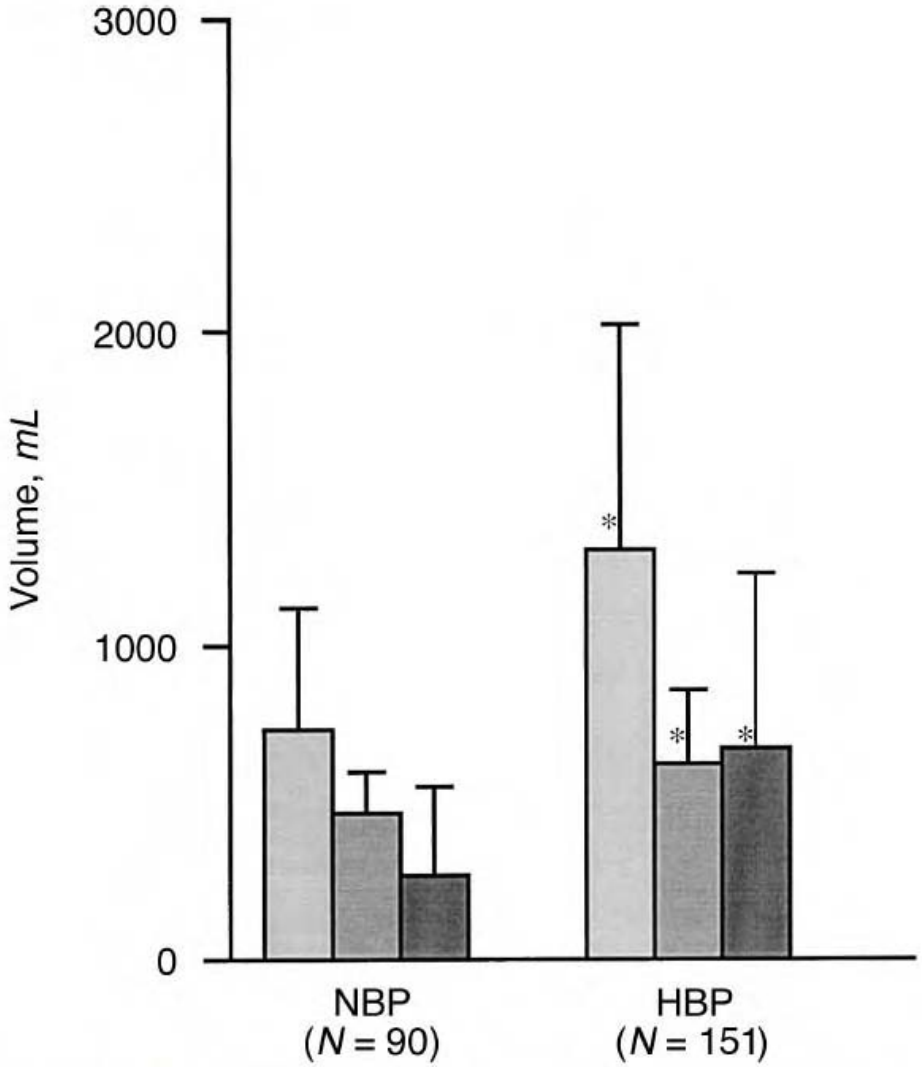
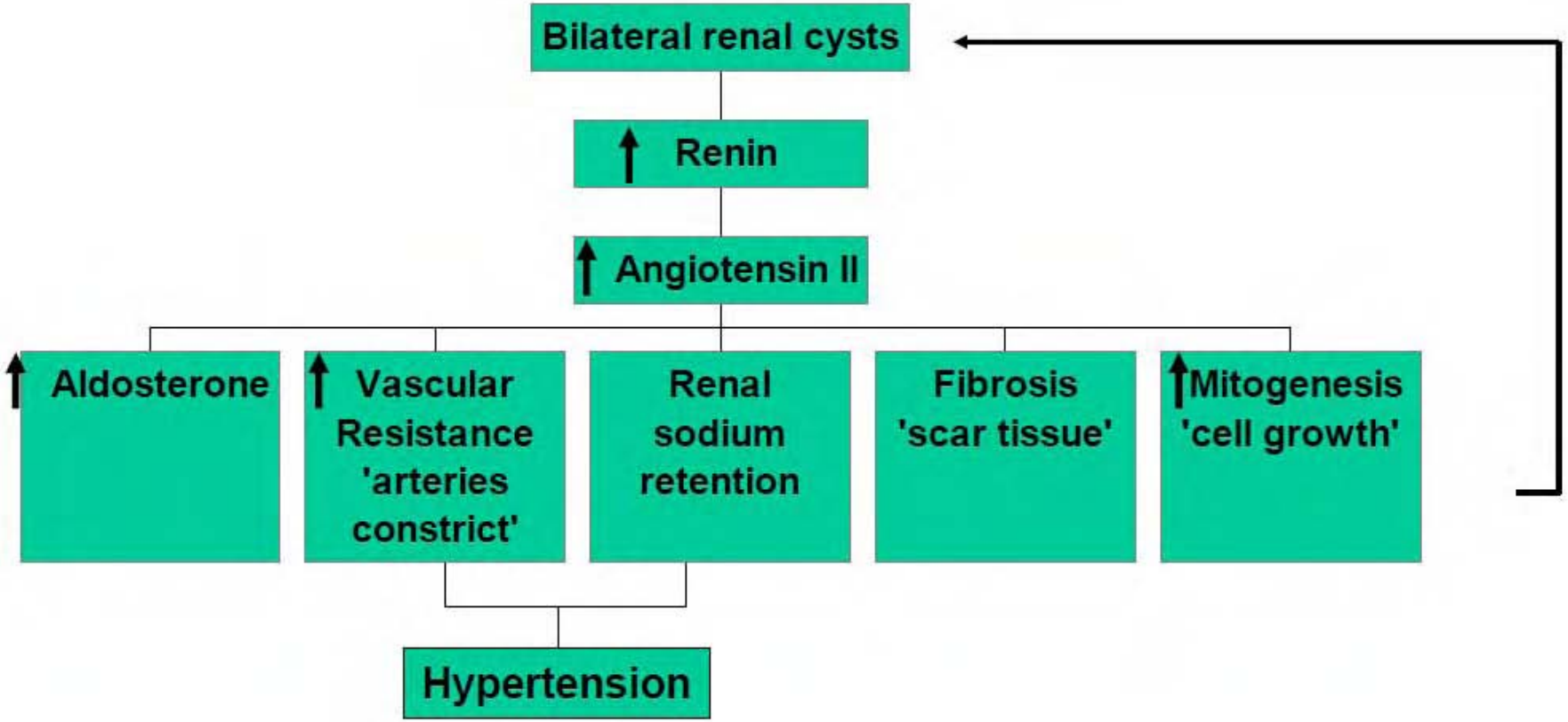


Fig. 4. Mean total renal, cystic, and non-cystic volumes in hypertensive (HBP) ($N = 151$) and normotensive (NBP) ($N = 90$) autosomal-dominant polycystic kidney disease (ADPKD) individuals. Total renal, cystic, and noncystic volumes were significantly greater in the hypertensive individuals (*) ($P < 0.0001$). Symbols are (□), total renal volume; (▩) noncystic volume; (■) cystic volume.

CRISP KI 64: 1035, 2003

Activation of the RAAS in PKD



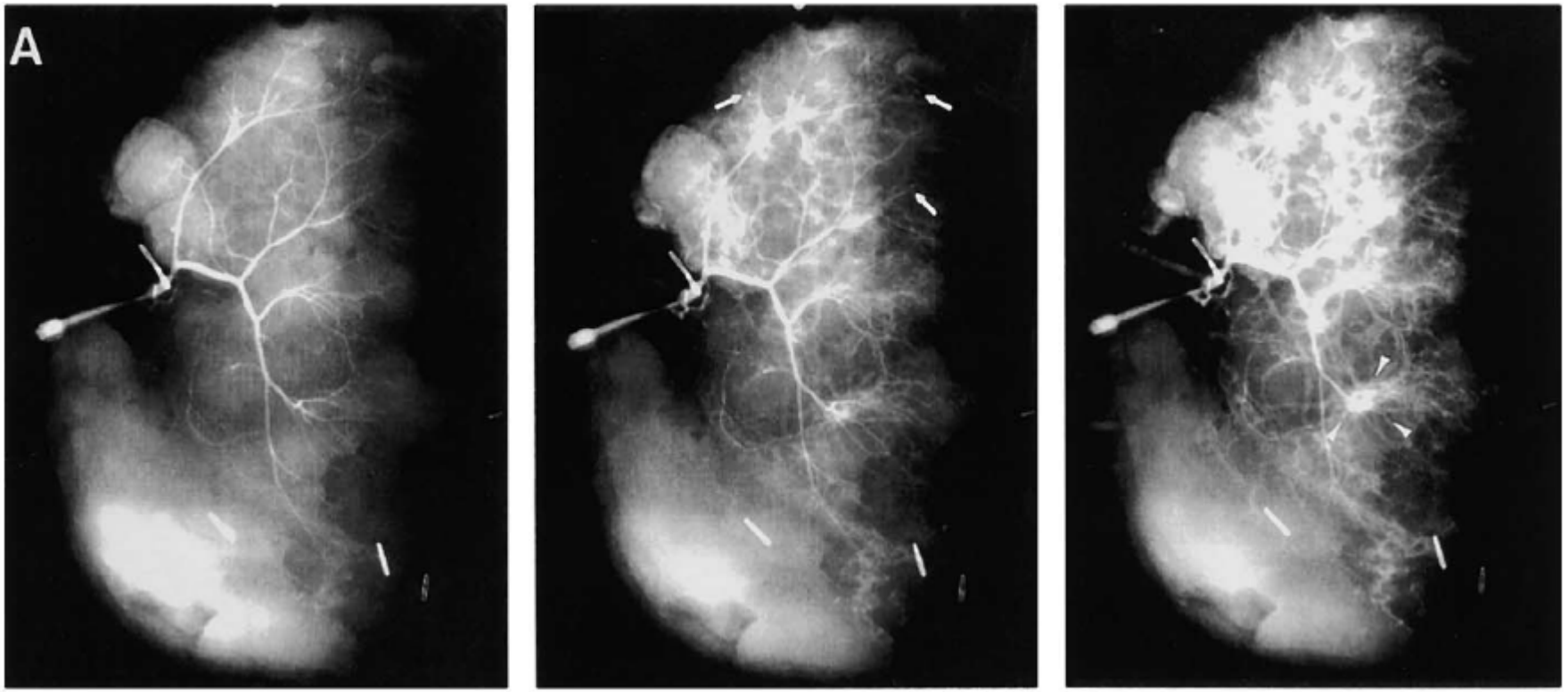
Hematuria in ADPKD

- Cyst hemorrhage occurs in ~60% of individuals
  - gross or microscopic hematuria if cyst connects to collecting system
  - intracyst or subcapsular hemorrhage without hematuria
- Excessive angiogenesis results in fragile blood vessels stretched across walls of enlarging cysts; susceptible to minor trauma with resultant hemorrhage
- Patients with recurrent episodes of gross hematuria have the largest kidneys and progress more quickly to kidney failure

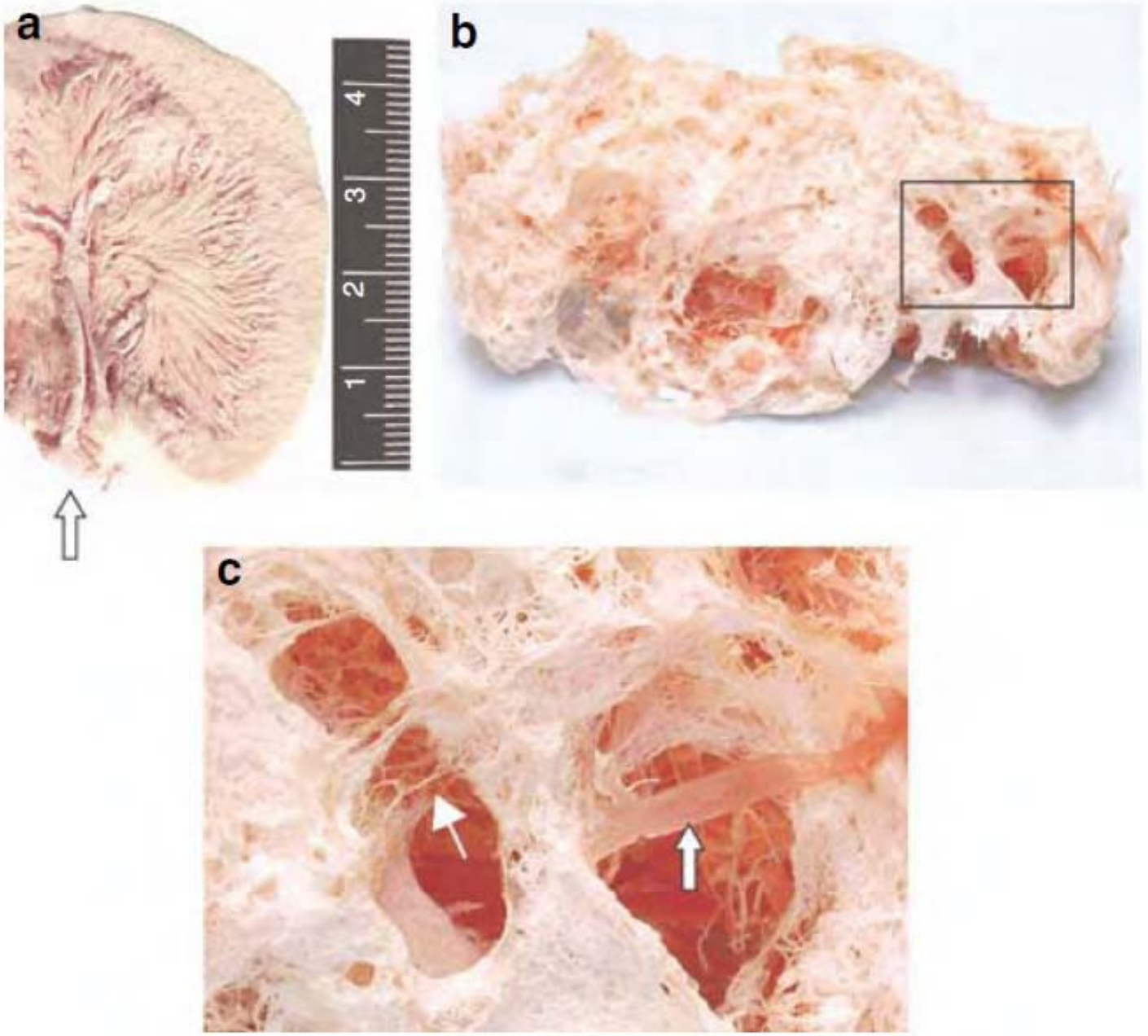
15 seconds

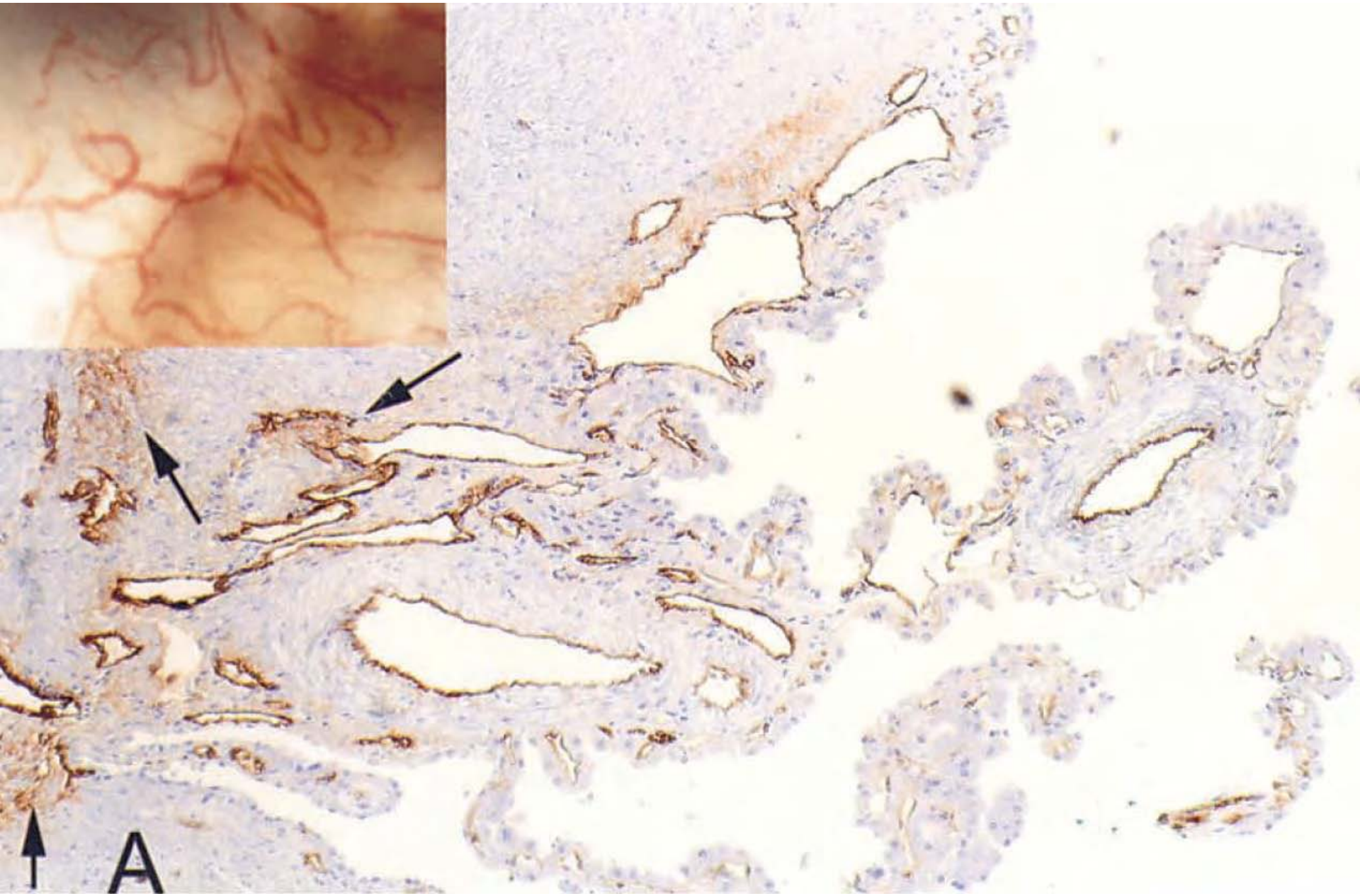
30 seconds

60 seco

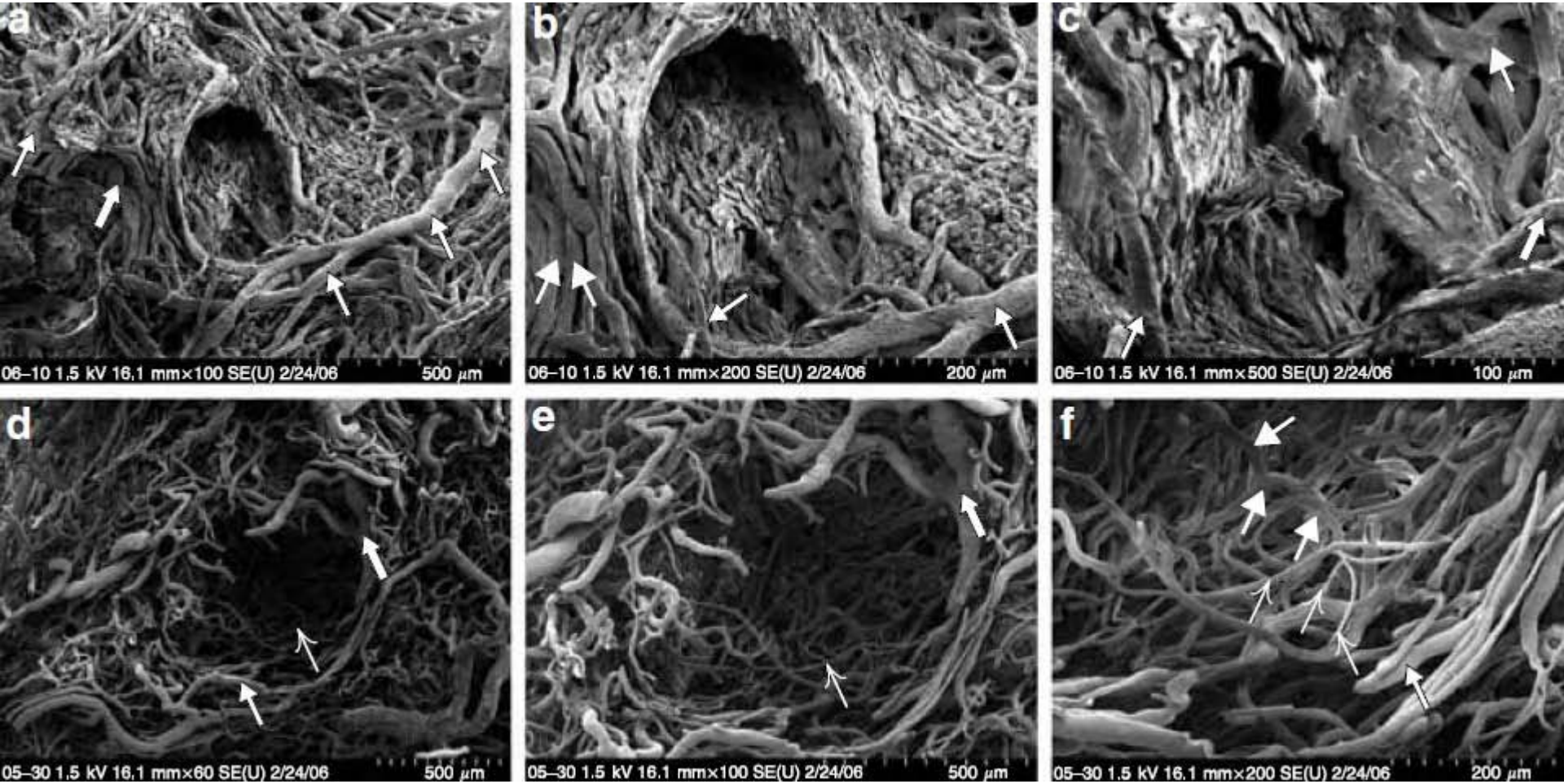


Bello-Reuss Kidney Int 60: 37, 2001


a
1
2
3
4
b
c



Bello-Reuss Kidney Int 60: 37, 2001


a
06–10 1.5 kV 16.1 mm×100 SE(U) 2/24/06 500 μm
b
06–10 1.5 kV 16.1 mm×200 SE(U) 2/24/06 200 μm
c
06–10 1.5 kV 16.1 mm×500 SE(U) 2/24/06 100 μm
d
05–30 1.5 kV 16.1 mm×60 SE(U) 2/24/06 500 μm
e
05–30 1.5 kV 16.1 mm×100 SE(U) 2/24/06 500 μm
f
05–30 1.5 kV 16.1 mm×200 SE(U) 2/24/06 200 μm

Treatment of Hematuria in ADPKD

- Appropriate diagnosis and treatment of specific entity, such as infection or stone
- Correction of coagulopathy, if present
- Conservative management with hydration, bed rest, and appropriate use of analgesics
- Rarely, massive bleeding may require transfusion, or kidney embolization or nephrectomy

Kidney Infection in ADPKD

- 30 to 50% of patients with ADPKD will have a urinary tract infection, either pyelonephritis or cyst infection, during their lifetime
- Urinary tract infections are more common in women with ADPKD
- Fever and flank pain are the presenting symptoms
- Urine culture may be negative in cyst infection, as cysts frequently don't communicate with the collecting system

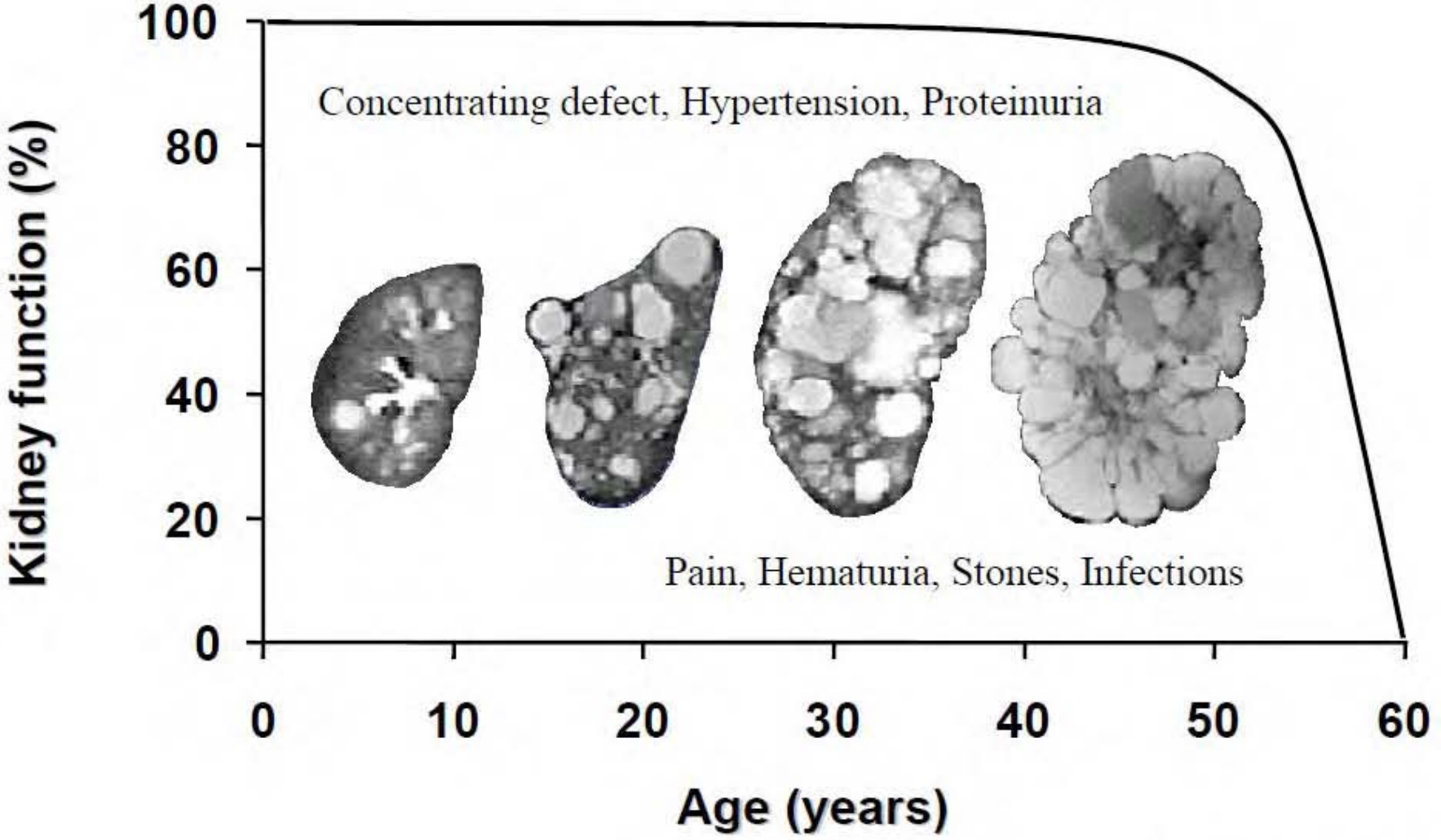
Treatment of Kidney Cyst Infection in ADPKD

- Lipophilic antibiotics such as ciprofloxacin, norfloxacin, trimethoprim, chloramphenicol
- Percutaneous or operative drainage is rarely needed; only for refractory infection
- Resistant organisms
- Localization of infected cyst is difficult
  - Labeled WBC or gallium scan
  - MRI with contrast
  - PET scan

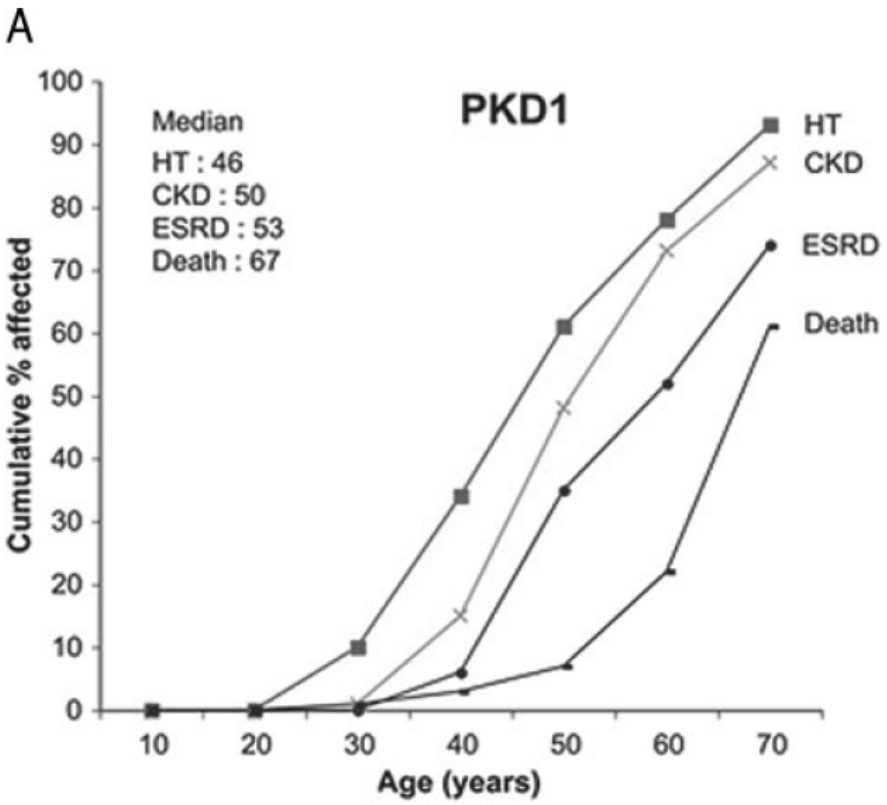
Nephrolithiasis in ADPKD

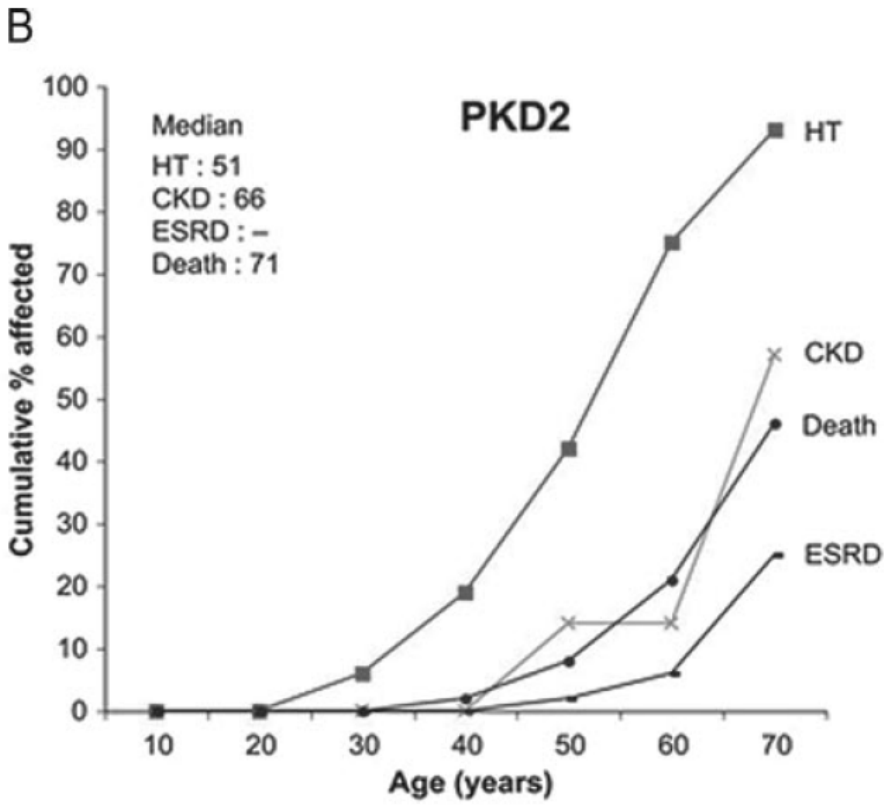
- Occurs in ~20% of patients
- Uric acid and/or calcium oxalate
- Predisposing factors include hypocitraturia, hyperoxaluria, hyperuricosuria, hyperuricemia, hypercalciuria, possible distal acidification defects
- Expanding cysts compress the collecting system producing urinary stasis, which predisposes to stone formation and infection

ADPKD Progression



Torres Mayo <aupCP1047707-9





Genotype, family and proteinuria are risk factors for renal events

Dicks et al, Clin J Am Soc Nephrol 1: 710, 2006

Progressive Loss of Kidney Function

- Rate of decline of GFR (data from MDRD study, starting at GFR $\leq$55 ml/min)

  Males 5 - 6 ml/min/year

  Females 4 - 5 ml/min/year

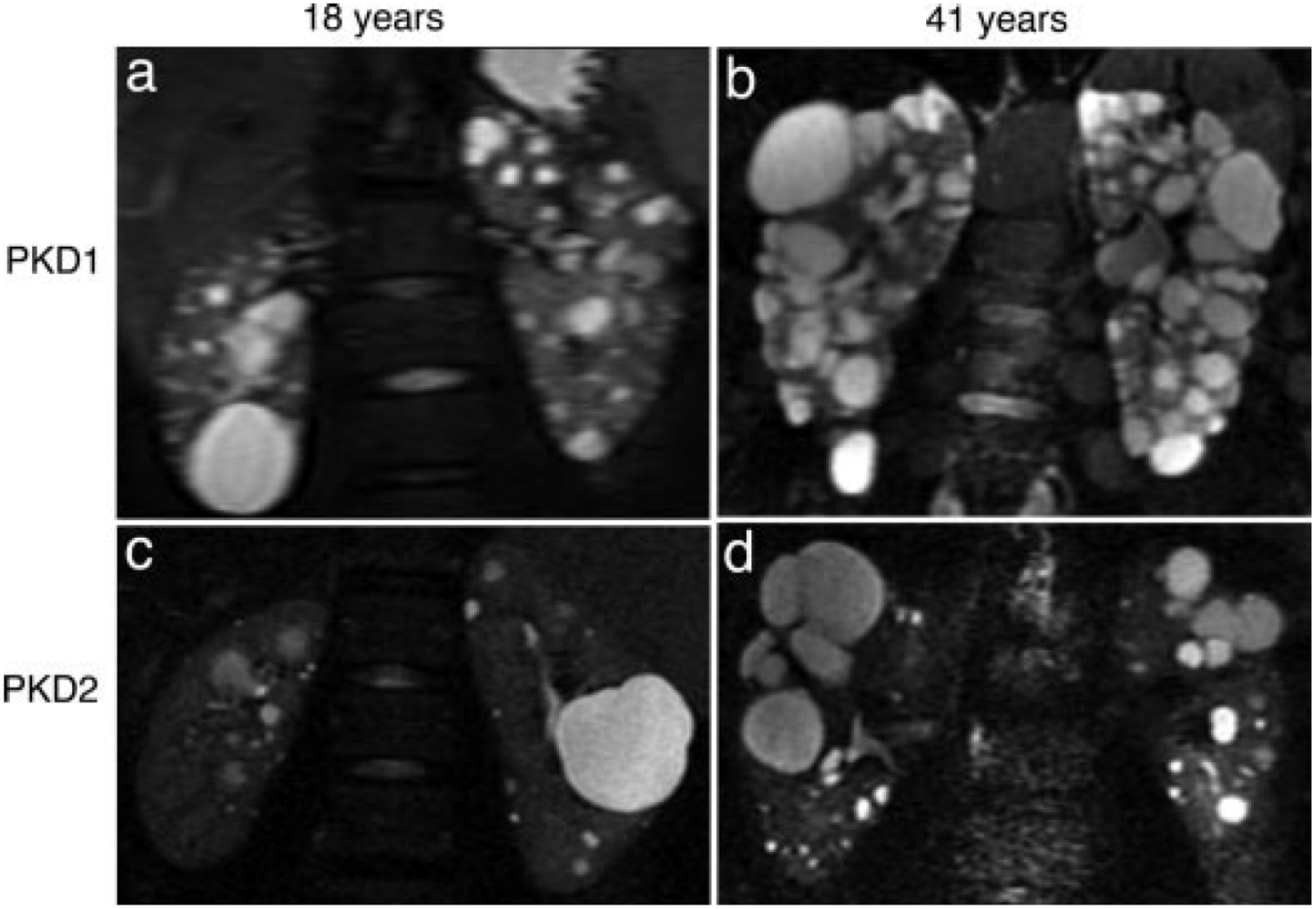
- Pattern of GFR loss has recently been established by CRISP study
  - **GFR stable for many years, despite progressive increase in total kidney volume**
  - **GFR decrease not detected until total kidney volume exceeds 1500 ml**

Consortium for Radiologic Imaging Studies in Polycystic Kidney Disease (CRISP)

- N=232 patients with early ADPKD without aztoaemia
- Renal enlargement occurs in a quantifiable, exponential manner and can be correlated directly with the decline in renal function (about 5% per year)
- The increase in kidney volume was about equal between right and left kidneys and was twice as fast in patients with the *PKD1* mutation compared to *PKD2* mutation
- A baseline total kidney volume >1500 ml was associated with a declining GFR
- In comparison with GFR, which declines very slowly in early stages of ADPKD and therefore is not a robust marker of disease progression, MRI assessment of renal volume seems to provide a promising tool for monitoring early disease progression and assessing the efficacy of therapeutic interventions

Grantham et al. *N Engl J Med* 354: 2122, 2006

Cyst Number but Not the Rate of Cystic Growth Is Associated with the Mutated Gene in Autosomal Dominant Polycystic Kidney Disease



Harris et al. *J Am Soc Nephrol 17*: 3013, 2006

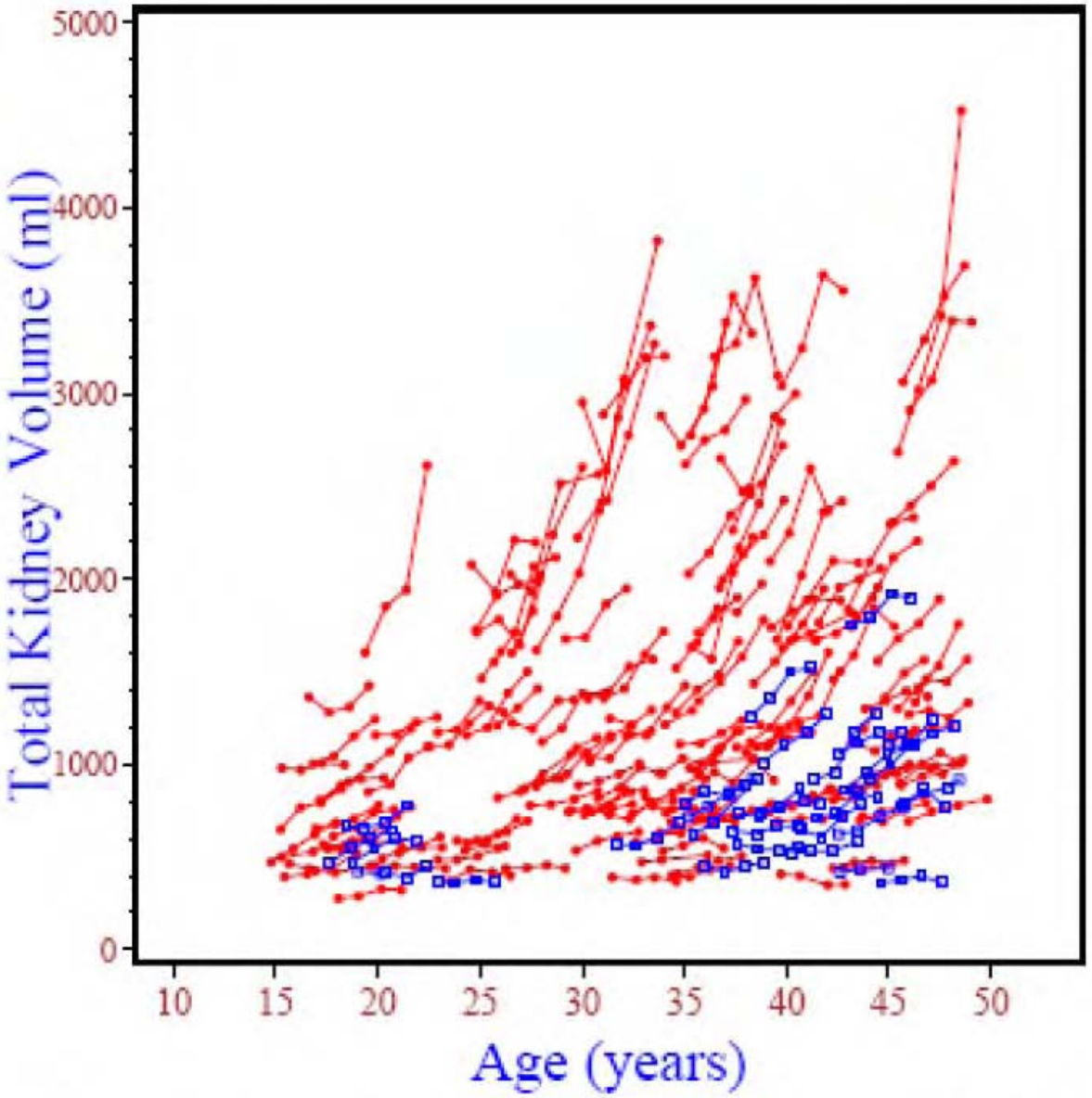
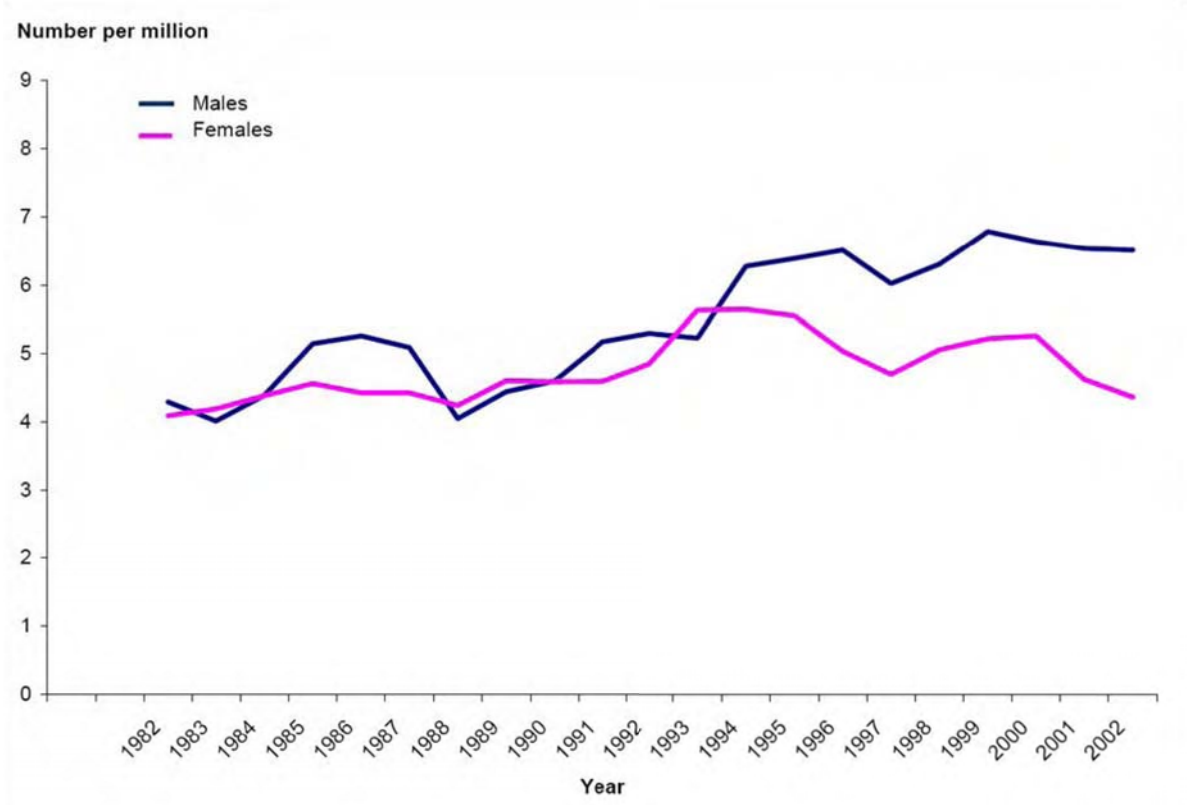


Figure 4. Distribution of PKD genotypes in relation to age. *PKD1* (red), *PKD2* (blue).

CRISP
NEJM 354:
2122-30

Trends in the incidence of ESRD due to PKD

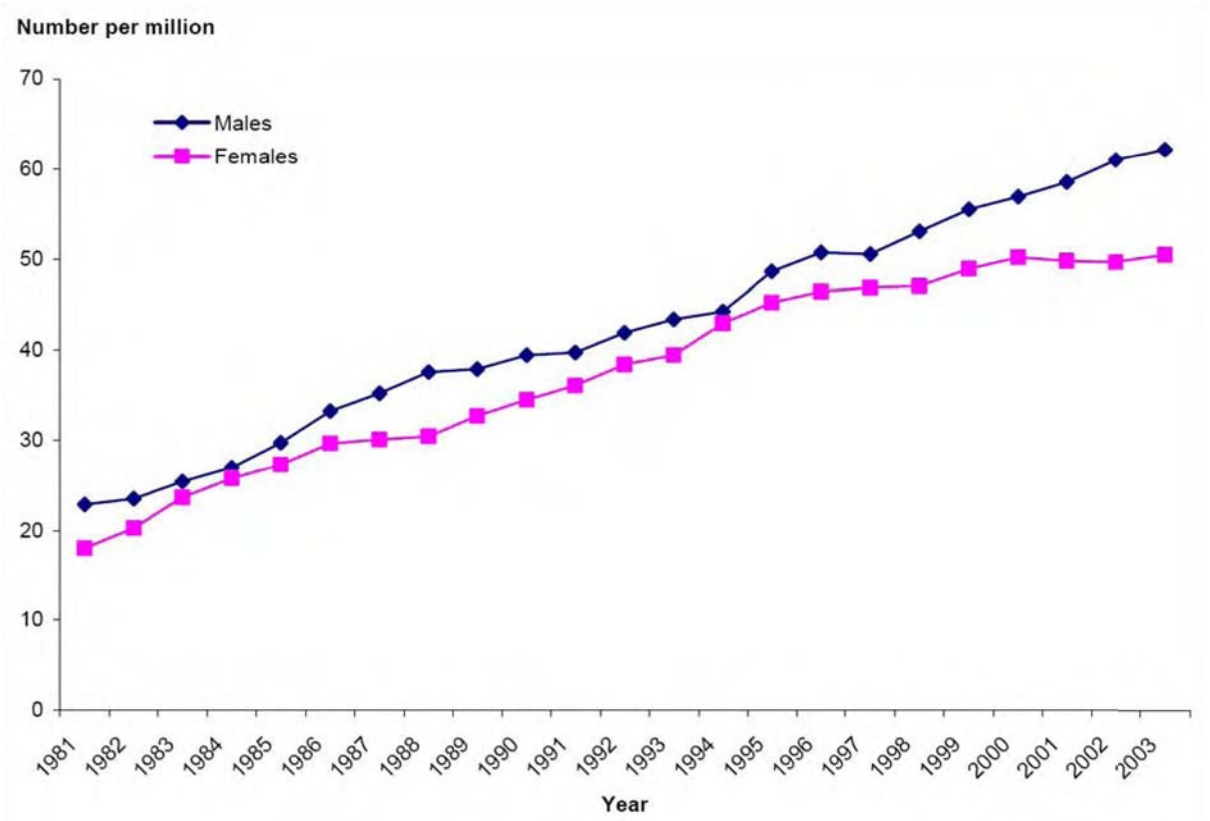


Notes

1. Age-standardised to the Australian population at 30 June 2001.
2. Data are three-year moving averages.

Source: AIHW analysis of ANZDATA Registry data.

Trends in prevalence of ESRD due to PKD



Note: Age-standardised to the Australian population at 30 June 2001.

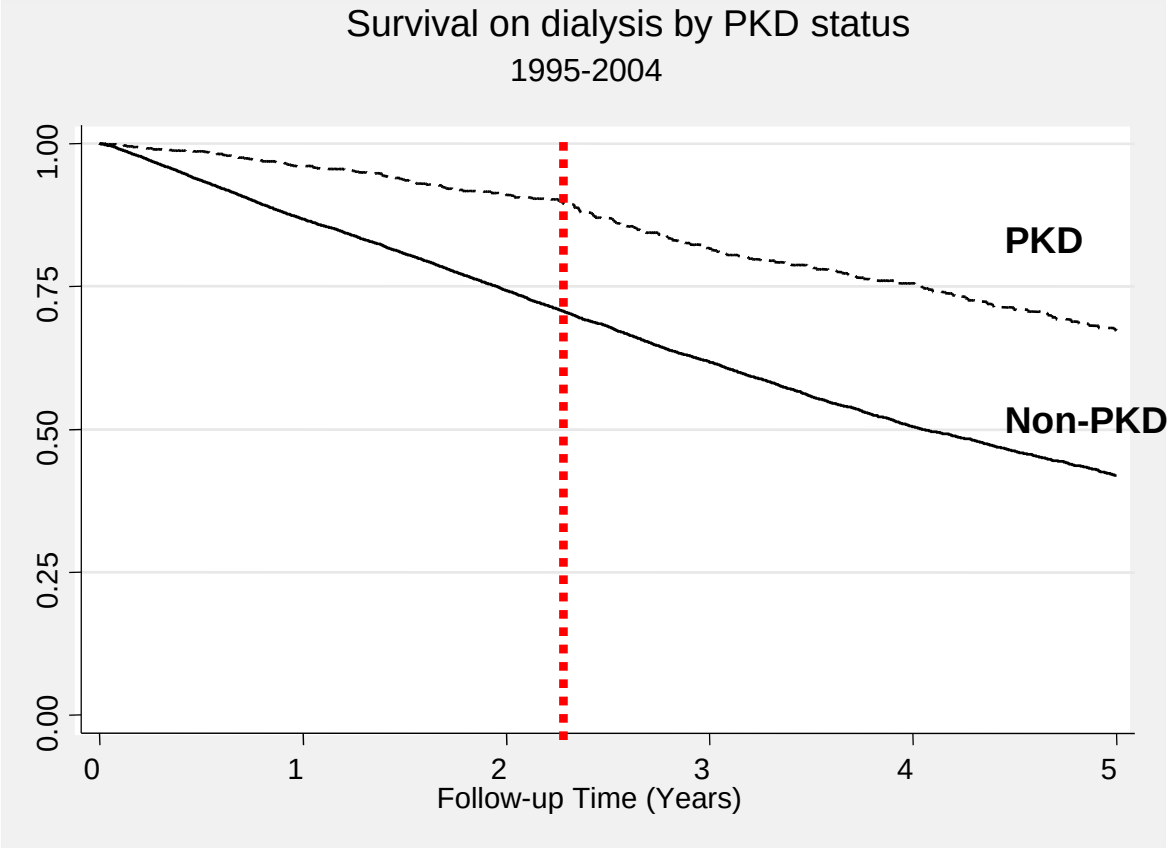
Source: AIHW analysis of ANZDATA Registry data.

End-stage renal failure due to ADPKD in Australia and New Zealand

5th most common cause of ESRF

Disease	2003	2004	2005	2006
Australia				
Glomerulonephritis	533 (27%)	493 (25%)	538 (24%)	539 (23%)
Analgesic Nephropathy	72 (4%)	47 (2%)	69 (3%)	52 (2%)
Polycystic Kidney	113 (5%)	127 (7%)	173 (7%)	147 (6%)
Reflux Nephropathy	74 (4%)	56 (3%)	65 (3%)	92 (4%)
Hypertension	300 (15%)	260 (13%)	332 (15%)	348 (15%)
Diabetic Nephropathy	515 (26%)	592 (30%)	718 (31%)	770 (32%)
Miscellaneous	236 (12%)	250 (13%)	254 (11%)	299 (13%)
Uncertain Diagnosis	140 (7%)	130 (7%)	134 (6%)	131 (5%)
Total	**1983 (100%)**	**1955 (100%)**	**2283 (100%)**	**2378 (100%)**
New Zealand				
Glomerulonephritis	117 (25%)	107 (24%)	101 (22%)	103 (21%)
Analgesic Nephropathy	-	2 (<1%)	1 (<1%)	1 (<1%)
Polycystic Kidney	22 (5%)	24 (5%)	33 (7%)	36 (7%)
Reflux Nephropathy	10 (2%)	12 (3%)	10 (2%)	14 (3%)
Hypertension	44 (10%)	72 (16%)	51 (11%)	58 (12%)
Diabetic Nephropathy	191 (41%)	185 (40%)	191 (42%)	202 (42%)
Miscellaneous	47 (10%)	30 (7%)	48 (11%)	37 (8%)
Uncertain Diagnosis	32 (7%)	25 (5%)	22 (5%)	33 (7%)
Total	**463 (100%)**	**457 (100%)**	**457 (100%)**	**484 (100%)**

ANZDATA report 2007



5-year survival is better in PKD patients. The survival advantage was most marked in the first 2 years (RR 0.31 for first 2 years *vs* 0.52 for the subsequent years).

Rangan GK, Shtangey, McDonald SP (in preparation)

Methods

- Sample consisted of all ANZ patients over the age of 20 and whose first treatment was dialysis. The characteristics of patients with or without ESRF due to PKD were analysed from the ANZ Dialysis and Transplantation Registry for the last 10 years (1995-2004);

- Diabetics were excluded from both PKD and non-PKD patients.

- Data for haemoglobin and EPO were analysed from 2000-2004

- Univariate and multivariate analyses were performed.

Age

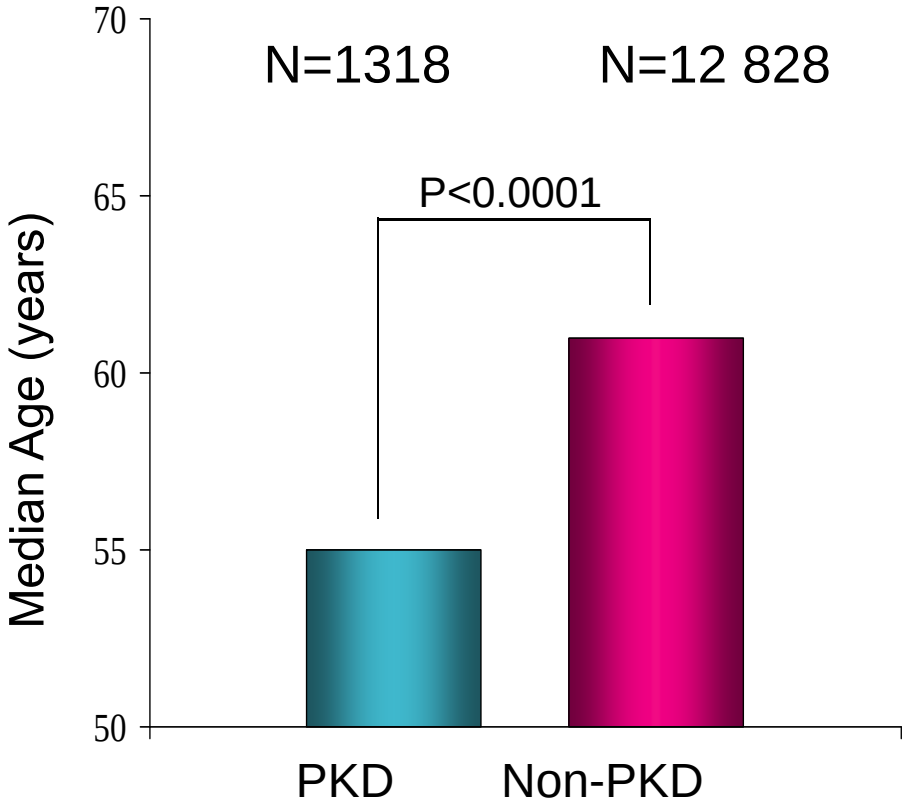


Figure 1. Patients with PKD are younger than non-PKD dialysis patients (55 *vs* 61 years of age).

Gender

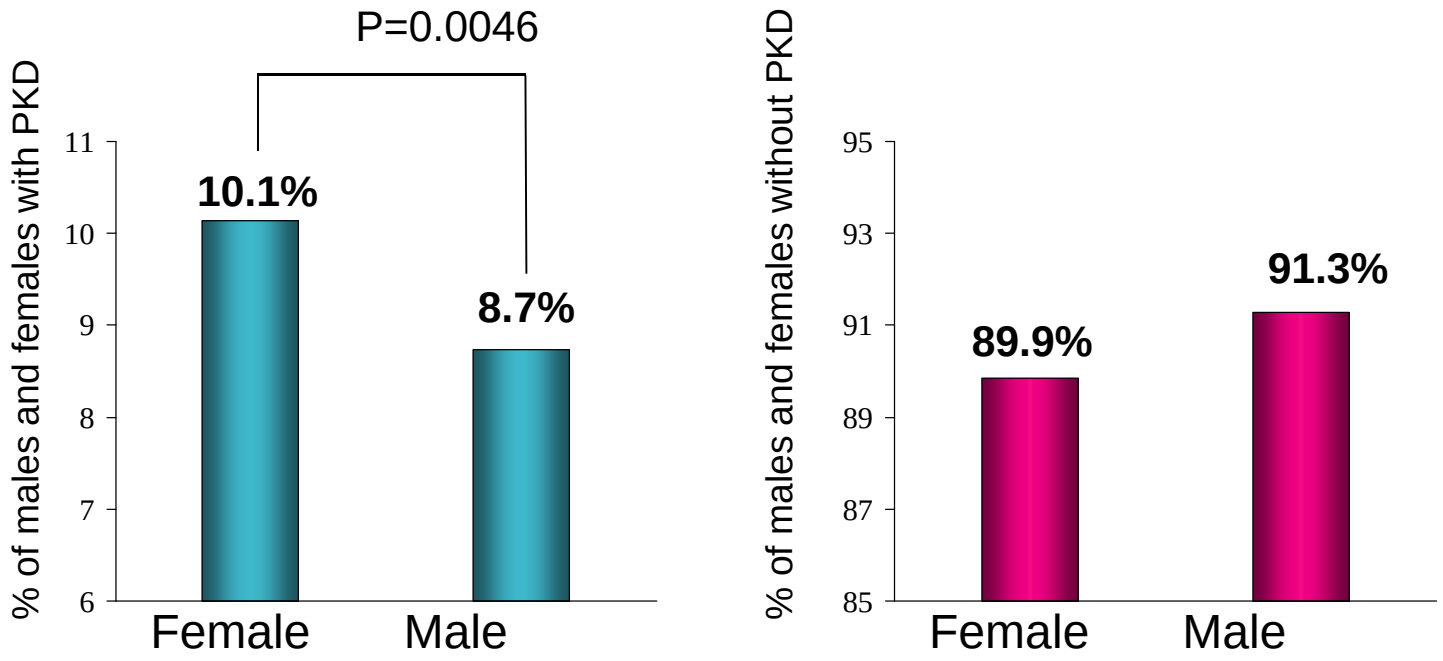


Figure 2. Men were less likely to have PKD as as a cause for ESRF (Odds ratio, **OR**: 0.85, P=0.0046).

Vascular disease

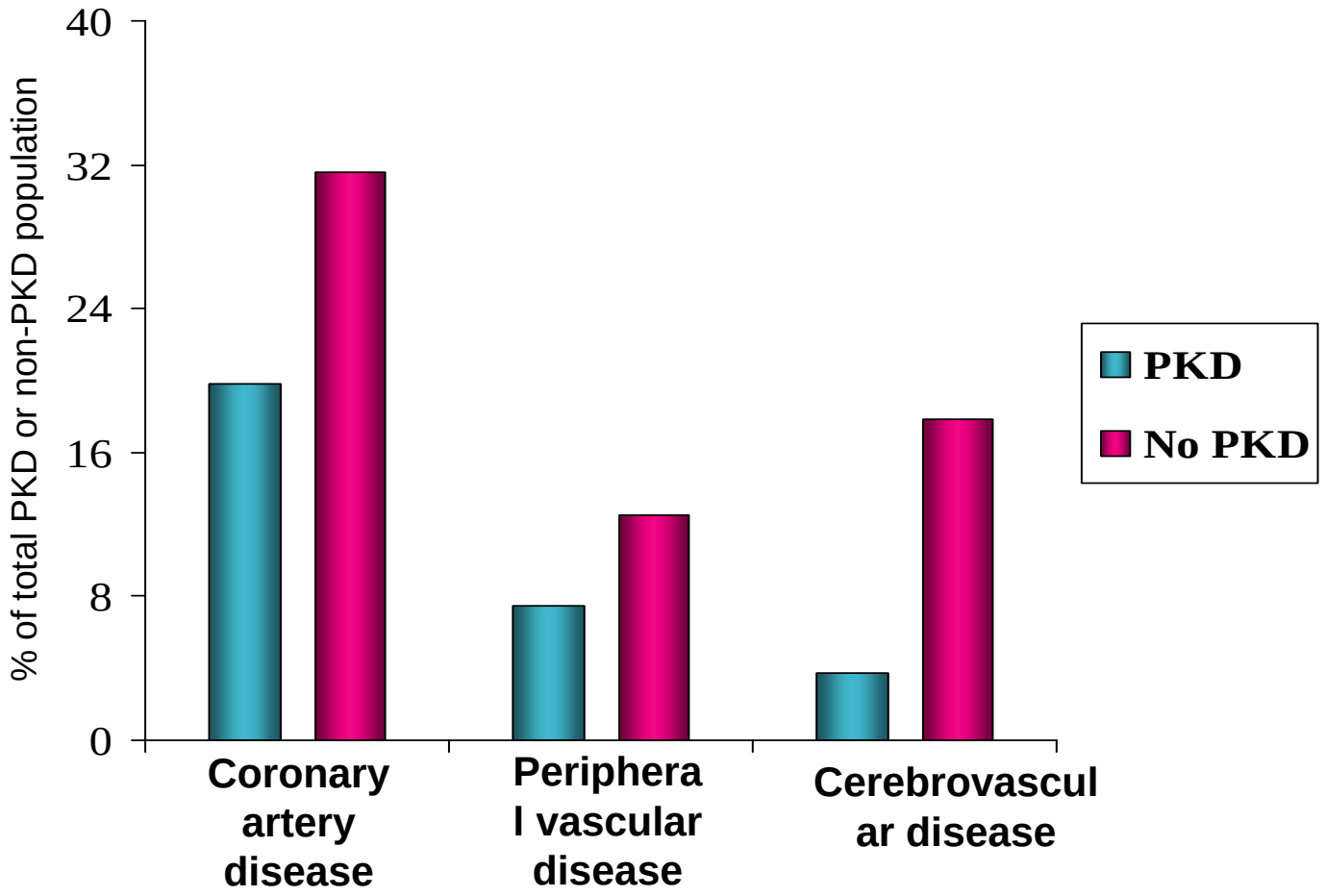


Figure 5. Vascular disease was reduced in PKD patients

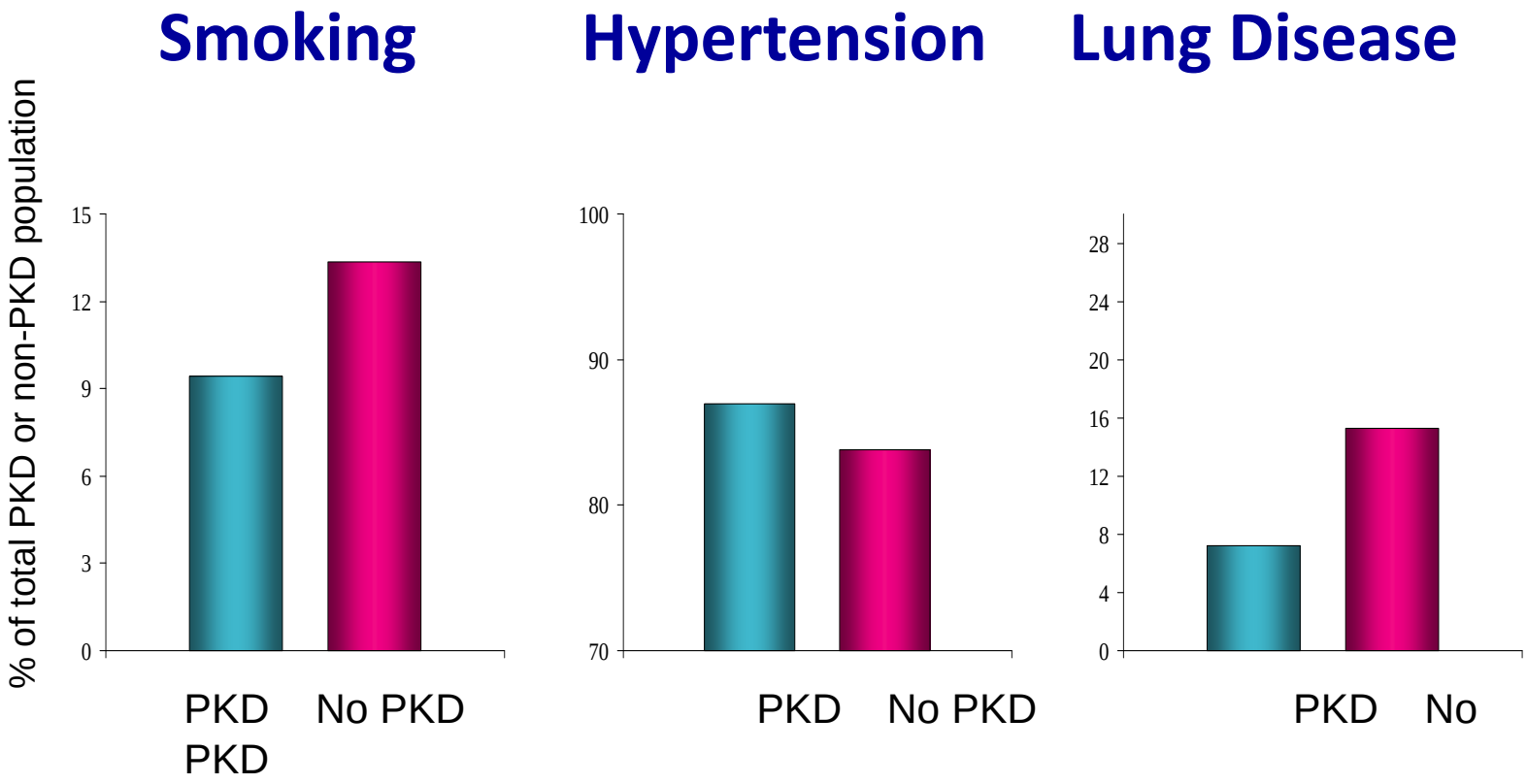


Figure 6. PKD patients smoked less and had reduced chronic lung disease, but hypertension was increased slightly.

Mean Hb is higher in PKD

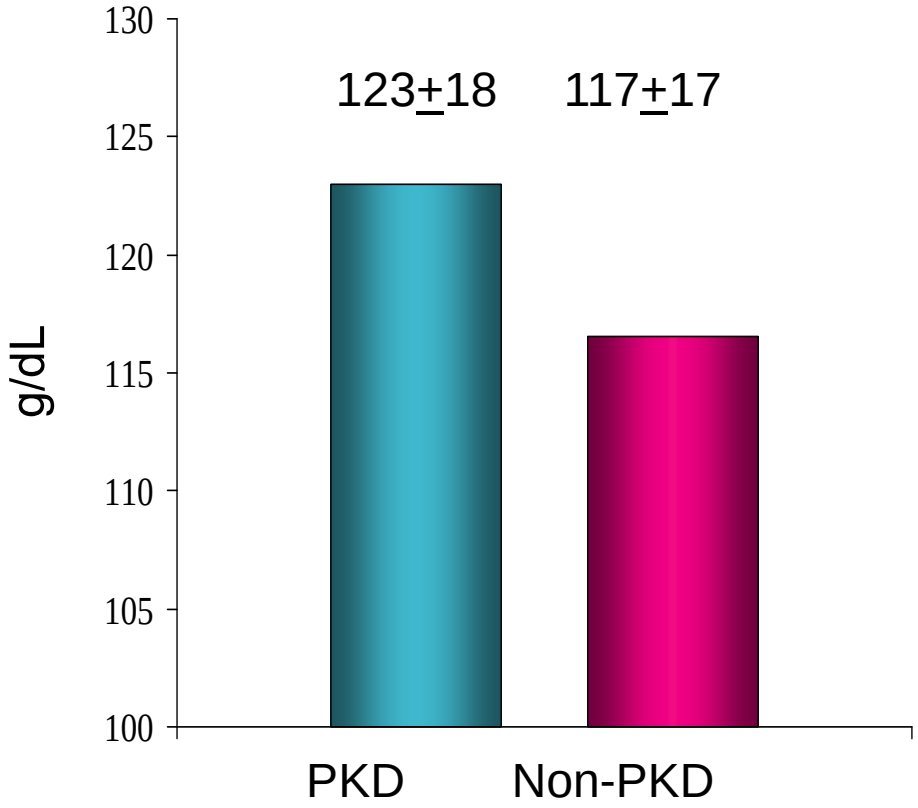


Figure 7. Patients with PKD have a higher Hb

Use of EPO is lower in PKD

- Among non-PKD patients, 11.89 % did not use and 88.11% used EPO agents
- Among PKD patients, 13.83% did not use and 73.16% used EPO agents. The difference was significant at p=0.001.
- PKD patients are less likely to use EPO agents than non-PKD patients, OR=0.37, 95% CI: [0.31; 0.44]. After adjusting for age, gender and race, PKD disease is still a significant predictor of EPO use.

Survival of PKD patients

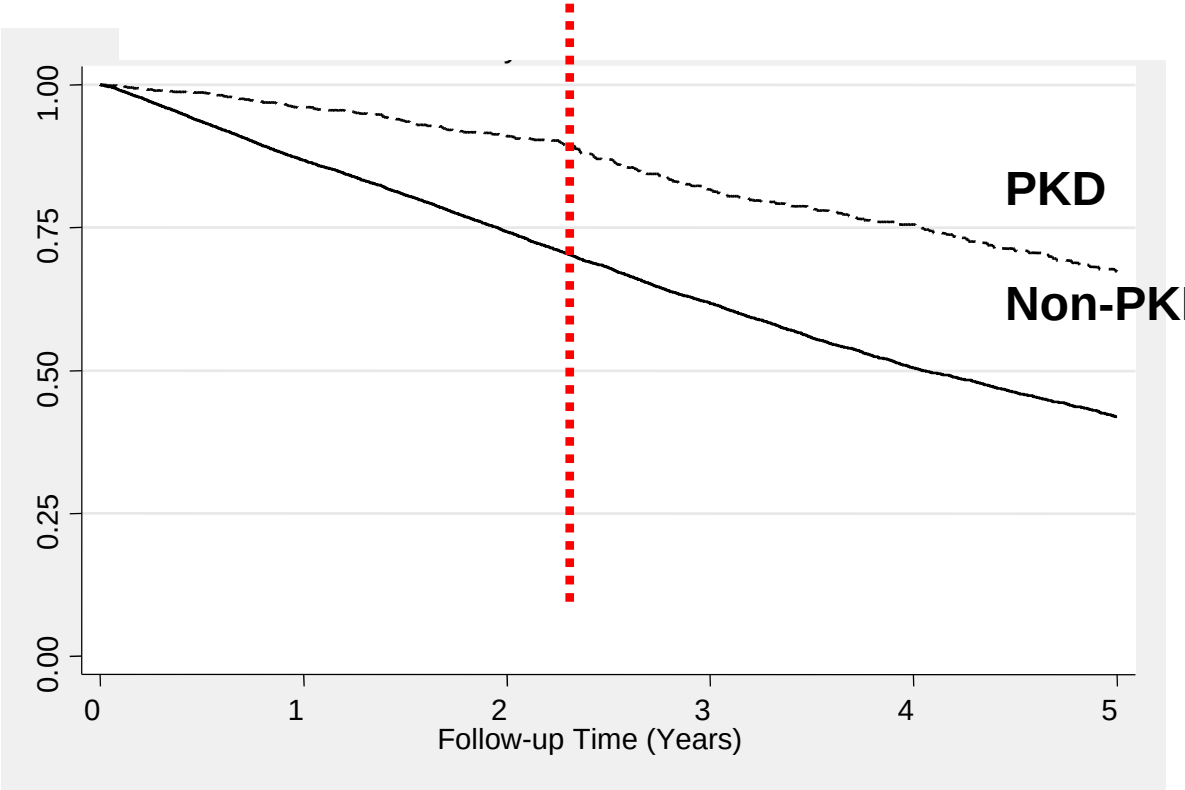


Figure 8. 5-year survival is better in PKD patients. The survival advantage was most marked in the first 2 years (RR 0.31 for first 2 years *vs* 0.52 for the subsequent years).

Principal Extrarenal Manifestations

- Hepatic and pancreatic cysts
  - Asymptomatic in many patients, but can expand and cause pain and infection; rarely massive PLD
- Cardiac valvular abnormalities
  - Mitral, tricuspid and aortic valve prolapse and regurgitation
- Intracranial aneurysms
  - Risk of rupture; found in approximately 5% of patients with no family history and about 22% of patients with family history of ICA or SAH
- Seminal vesicle cysts
  - Found in ~39-60% of men; undefined risk of infertility

Treatment of ADPKD (1)

- There is no specific therapy
- Pain
  - Differential diagnosis: bleed vs. infection vs. obstruction vs. stone
  - Analgesics
  - Percutaneous drainage; laparoscopic or surgical unroofing of individual cysts
- Infection: lipophilic antibiotics
- Hypertension
  - ACE inhibitors thought to be beneficial

Treatment of ADPKD (2)

- Progressive kidney insufficiency
  - Lack of proven benefit of low protein diets or ACE-I
  - Cyst decompression does not alter progression
  - Renal replacement therapy
- Extrarenal manifestations
  - Intervene as needed for symptoms
  - Screen for cerebral aneurysms with + family history; antibiotic prophylaxis for valvular regurgitation
  - Avoid estrogen/progesterone in women (effect on liver cyst disease)

Clinical Trials

HALT PKD

A Clinical Research Study

To

HALT Progression of Polycystic Kidney Disease

Developed by the Polycystic Kidney Disease Treatment Network

www.pkd.wustl.edu/pkdtn

Sponsored by
The National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK)
The National Institutes of Health (NIH)
U.S. Department of Health and Human Services

Overall aim: The efficacy of interruption of the renin-angiotensin-aldosterone system (RAAS) on the progression of cystic disease and on the decline in renal function in autosomal dominant kidney disease (ADPKD) will be assessed.

Study Design: Two concurrent multi-centre randomized, double-blind, placebo control clinical trials targeting different levels of kidney function:

STUDY A: early disease defined by GFR >60 mL/min/1.73 m2 with primary outcome as change in total kidney volume, as assessed by abdominal MR at baseline, 2 years and 4 years followup; and

STUDY B: moderately advanced disease defined by GFR 30-60 mL/min/1.73 m2 with primary outcome as time to the 50% reduction of baseline eGFR, ESRD (initiation of dialysis or preemptive transplant) or death, followed for 4-6 years with average length of followup being 5 years.

Participants will be recruited and enrolled, either to Study A or B, over the first two years.

Total enrollment: 1018 patients in U.S. centres only

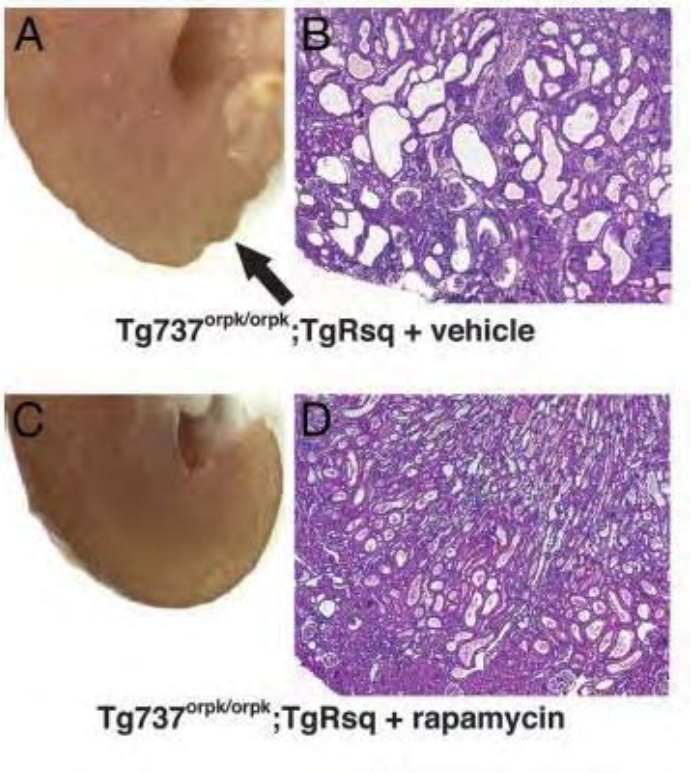
Study timetable: Start January 2006 and expected completion: December 2011

Sponsors: NIH, Ingelheim Pharmaceuticals, Merck, PKD Foundation

www.clinicaltrials.gov accessed May 9th 2007

Sirolimus

- Clinical trials in humans to test the efficacy of various agents in PKD are currently underway. One such agent is sirolimus
- Sirolimus is an immunosuppressant drug with anti-proliferative effects. It is known to inhibit mTOR, an enzyme involved in mRNA translation that is upregulated in PKD
- Sirolimus given to ADPKD rats inhibits cystogenesis via its anti-proliferative effects.
- Evidence suggest it may have other mechanisms of action that relate to angiogenesis



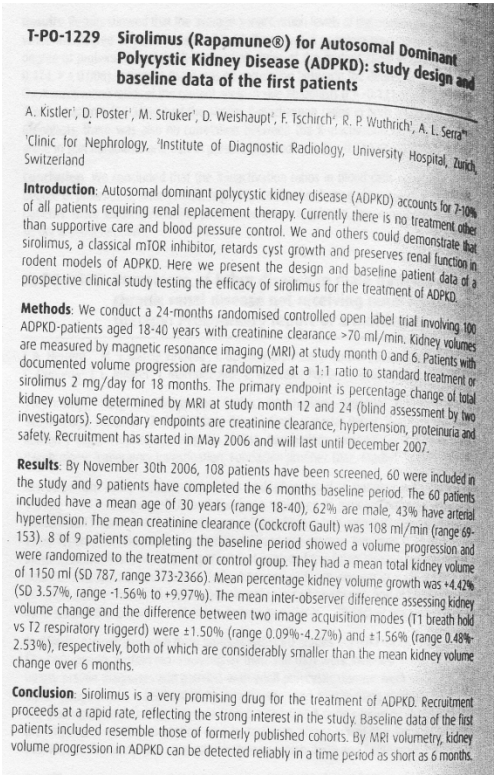
Shillingford et al. *PCNAS* (2006) 103, 5466-71

T-PO-1229: Sirolimus for ADPKD: study design and baseline data of the first patients

A. Kistler, D. Poster, M. Struker, D. Weishaupt, F. Tschirch, RP Wuthrich, AL Serra.
Clinic for Nephrology, Institute of Diagnostic Radiology, University Hospital, Zurich, Switzerland

Proceedings of the World Congress of Nephrology, April 21-25th 2007, Rio de Janeiro, Brazil (page 402)

- **Design:** Prospective RCT open-label trial involving. Kidney volume by MRI at study month 0 and 6. Patients with documented progression are randomized 1:1 ratio to a standard treatment or sirolimus 2 mg/day for 18 months. Recruitment started May 2006 and will last December 2007
- **Inclusion criteria:** 100 ADPKD-patients aged 18-40 years with CrCl >70ml/min before screening
- **Outcome measures:** Primary endpoint is percentage change of total kidney volume determined by MRI at study month 12 and 24. Secondary endpoints are CrCl, proteinuria, hypertension and safety.
- **Results:** By November 2006, 108 patients screened, 60 were included in the study and 9 patients have completed the 6 months baseline period. The 60 patients have a mean age of 30 (18-40), 62% were male, 43% have hypertension, mean CrCl was 108 ml/min (range 69-153). 8 of the 9 patients completing the baseline period showed volume progression and were randomized to the treatment or the control group. The mean total kidney volume was 1150 ml.
- **Conclusion:** Recruitment rapid; MRI volumetry can be detected reliably.



T-PO-1229 Sirolimus (Rapamune®) for Autosomal Dominant Polycystic Kidney Disease (ADPKD): study design and baseline data of the first patients

A. Kistler[1], D. Poster[1], M. Struker[1], D. Weishaupt[2], F. Tschirch[2], R. P. Wuthrich[1], A. L. Serra[1]*

[1]Clinic for Nephrology, [2]Institute of Diagnostic Radiology, University Hospital, Zurich, Switzerland

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) accounts for 7-10% of all patients requiring renal replacement therapy. Currently there is no treatment other than supportive care and blood pressure control. We and others could demonstrate that sirolimus, a classical mTOR inhibitor, retards cyst growth and preserves renal function in rodent models of ADPKD. Here we present the design and baseline patient data of a prospective clinical study testing the efficacy of sirolimus for the treatment of ADPKD.

Methods: We conduct a 24-months randomised controlled open label trial involving 100 ADPKD-patients aged 18-40 years with creatinine clearance >70 ml/min. Kidney volumes are measured by magnetic resonance imaging (MRI) at study month 0 and 6. Patients with documented volume progression are randomized at a 1:1 ratio to standard treatment or sirolimus 2 mg/day for 18 months. The primary endpoint is percentage change of total kidney volume determined by MRI at study month 12 and 24 (blind assessment by two investigators). Secondary endpoints are creatinine clearance, hypertension, proteinuria and safety. Recruitment has started in May 2006 and will last until December 2007.

Results: By November 30th 2006, 108 patients have been screened, 60 were included in the study and 9 patients have completed the 6 months baseline period. The 60 patients included have a mean age of 30 years (range 18-40), 62% are male, 43% have arterial hypertension. The mean creatinine clearance (Cockcroft Gault) was 108 ml/min (range 69-153). 8 of 9 patients completing the baseline period showed a volume progression and were randomized to the treatment or control group. They had a mean total kidney volume of 1150 ml (SD 787, range 373-2366). Mean percentage kidney volume growth was +4.42% (SD 3.57%, range -1.56% to +9.97%). The mean inter-observer difference assessing kidney volume change and the difference between two image acquisition modes (T1 breath hold vs T2 respiratory triggerd) were ±1.50% (range 0.09%-4.27%) and ±1.56% (range 0.48%-2.53%), respectively, both of which are considerably smaller than the mean kidney volume change over 6 months.

Conclusion: Sirolimus is a very promising drug for the treatment of ADPKD. Recruitment proceeds at a rapid rate, reflecting the strong interest in the study. Baseline data of the first patients included resemble those of formerly published cohorts. By MRI volumetry, kidney volume progression in ADPKD can be detected reliably in a time period as short as 6 months.

Potential pitfalls in designing experimental studies in PKD: Sirolimus as a case example

2002+: Animal models consistently showed sirolimus consistently reduced cyst size

Table 4
Effects of sirolimus in experimental models of polycystic kidney disease and tuberous sclerosis.

Species	Model	TOR inhibitor	Effect	Author
Rat	Eker	S	Reduced renal tumours	(Kenerson et al., 2002)
Rat	Eker	S	Reduced renal tumours	(Kenerson et al., 2005)
Rat	Han:Sprd	S	Reduced cyst formation	(Tao et al., 2005)
Rat	Han:Sprd	E	Reduced cyst formation	(Wu et al., 2007)
Rat	Han:Sprd	E	Reduced cyst formation	(Berthier et al., 2007)
Mice	Tg737	S	Reduced cyst formation	(Shillingford et al., 2006)
Mice	bpk	S	Reduced cyst formation	(Shillingford et al., 2006)
Rats	Han:Sprd	S	Reduced cyst formation	(Wahl et al., 2006)

Rangan, Burgess, Schwensen, Harris et al. 2009

June 2010: Results of first clinical tria not so positive


THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Sirolimus and Kidney Growth in Autosomal Dominant Polycystic Kidney Disease

Andreas L. Serra, M.D., Diane Poster, M.D., Andreas D. Kistler, M.D., Fabienne Krauer, B.S., Shagun Raina, M.S., James Young, Ph.D., Katharina M. Rentsch, Ph.D., Katharina S. Spanaus, M.D., Oliver Senn, M.D., M.P.H., Paulus Kristanto, Ph.D., Hans Scheffel, M.D., Dominik Weishaupt, M.D., and Rudolf P. Wüthrich, M.D.

ABSTRACT

BACKGROUND
In autosomal dominant polycystic kidney disease (ADPKD), aberrant activation of the mammalian target of rapamycin (mTOR) pathway is associated with progressive kidney enlargement. The drug sirolimus suppresses mTOR signaling.

METHODS
In this 18-month, open-label, randomized, controlled trial, we sought to determine whether sirolimus halts the growth in kidney volume among patients with ADPKD. We randomly assigned 100 patients between the ages of 18 and 40 years to receive either sirolimus (target dose, 2 mg daily) or standard care. All patients had an estimated creatinine clearance of at least 70 ml per minute. Serial magnetic resonance imaging was performed to measure the volume of polycystic kidneys. The primary outcome was total kidney volume at 18 months on blinded assessment. Secondary outcomes were the glomerular filtration rate and urinary albumin excretion rate at 18 months.

RESULTS
At randomization, the median total kidney volume was 907 cm^3 (interquartile range, 577 to 1330) in the sirolimus group and 1003 cm^3 (interquartile range, 574 to 1422) in the control group. The median increase over the 18-month period was 99 cm^3 (interquartile range, 43 to 173) in the sirolimus group and 97 cm^3 (interquartile range, 37 to 181) in the control group. At 18 months, the median total kidney volume in the sirolimus group was 102% of that in the control group (95% confidence interval, 99 to 105; P=0.26). The glomerular filtration rate did not differ significantly between the two groups; however, the urinary albumin excretion rate was higher in the sirolimus group.

CONCLUSIONS
In adults with ADPKD and early chronic kidney disease, 18 months of treatment with sirolimus did not halt polycystic kidney growth. (ClinicalTrials.gov number, NCT00346918.)


ORIGINAL ARTICLE

Everolimus in Patients with Autosomal Dominant Polycystic Kidney Disease

Gerd Walz, M.D., Klemens Budde, M.D., Marwan Mannaa, M.D., Jens Nürnberger, M.D., Christoph Wanner, M.D., Claudia Sommerer, M.D., Ulrich Kunzendorf, M.D., Bernhard Banas, M.D., Walter H. Hörl, M.D., Ph.D., Nicholas Obermüller, M.D., Wolfgang Arns, M.D., Hermann Pavenstädt, M.D., Jens Gaedeke, M.D., Martin Büchert, Ph.D., Christoph May, Ph.D., Harald Gschaidmeier, Ph.D., Stefan Kramer, Ph.D., and Kai-Uwe Eckardt, M.D.

ABSTRACT

BACKGROUND
Autosomal dominant polycystic kidney disease (ADPKD) is a slowly progressive hereditary disorder that usually leads to end-stage renal disease. Although the underlying gene mutations were identified several years ago, efficacious therapy to curtail cyst growth and prevent renal failure is not available. Experimental and observational studies suggest that the mammalian target of rapamycin (mTOR) pathway plays a critical role in cyst growth.

METHODS
In this 2-year, double-blind trial, we randomly assigned 433 patients with ADPKD to receive either placebo or the mTOR inhibitor everolimus. The primary outcome was the change in total kidney volume, as measured on magnetic resonance imaging, at 12 and 24 months.

RESULTS
Total kidney volume increased between baseline and 1 year by 102 ml in the everolimus group, versus 157 ml in the placebo group (P=0.02) and between baseline and 2 years by 230 ml and 301 ml, respectively (P=0.06). Cyst volume increased by 76 ml in the everolimus group and 98 ml in the placebo group after 1 year (P=0.27) and by 181 ml and 215 ml, respectively, after 2 years (P=0.28). Parenchymal volume increased by 26 ml in the everolimus group and 62 ml in the placebo group after 1 year (P=0.009) and by 56 ml and 93 ml, respectively, after 2 years (P=0.11). The mean decrement in the estimated glomerular filtration rate after 24 months was 8.9 ml per minute per 1.73 m^2 of body-surface area in the everolimus group versus 7.7 ml per minute in the placebo group (P=0.15). Drug-specific adverse events were more common in the everolimus group; the rate of infection was similar in the two groups.

CONCLUSIONS
Within the 2-year study period, as compared with placebo, everolimus slowed the increase in total kidney volume of patients with ADPKD but did not slow the decline in progressive renal impairment. (EudraCT number, 2006-001485-16; ClinicalTrials .gov number, NCT00414440.)

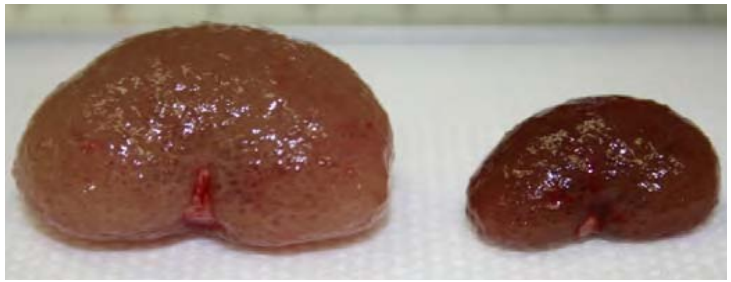
CONCLUSIONS
In adults with ADPKD and early chronic kidney disease, 18 months of treatment with sirolimus did not halt polycystic kidney growth. (ClinicalTrials.gov number, NCT00346918.)

CONCLUSIONS
Within the 2-year study period, as compared with placebo, everolimus slowed the increase in total kidney volume of patients with ADPKD but did not slow the decline in progressive renal impairment. (EudraCT number, 2006-001485-16; ClinicalTrials .gov number, NCT00414440.)

Designing experiments: Timing is everything - Sirolimus as a case example

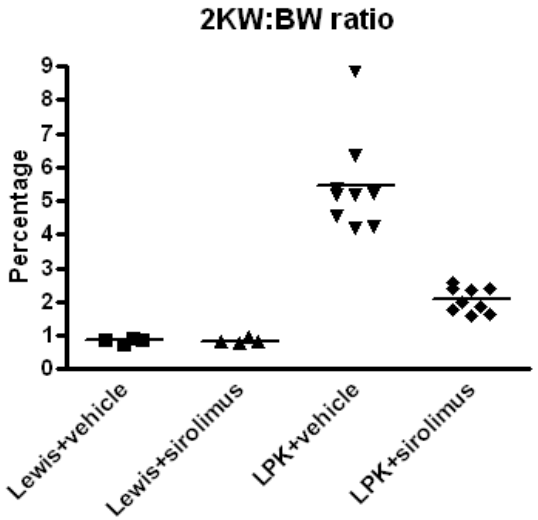
Starting treatment before maximal kidney growth (week 3 to 10)

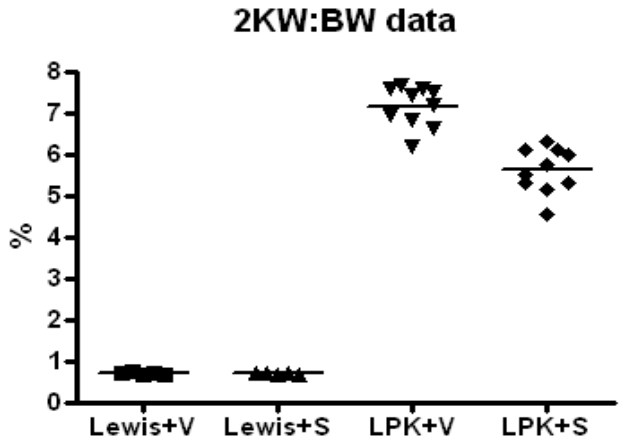
Starting treatment when maximal kidney growth was present (week 10 to 20)



Vehicle R_x Sirolimus R_x

Vehicle R_x Sirolimus R_x





Schwensen et al. 201

Vasopressin V2 receptor antagonists (tolvaptan)

Reduce cAMP levels

Phase III clinical trials underway

Including 7 centres in Australia

OPC-31260 Vasopressin Blockade Pkd2$^{WS25/-}$ mice
Kidneys at 16 weeks (treated 13 weeks)

	Control	OPC-31260
Histologic Section	A	B
Gross Appearance	C	D

Octreotide

- Somatastatin acting on SST2 receptors inhibits cAMP
- Octreotide (somatostatin analogue)
- Clinical trial in Italy

Stage	I. (Subclinical)	II. (Early stage)	III. (Late stage)
Clinical description	Gene carriers, variable cyst number and size, normal blood pressure, normal kidney function (>90 ml/min or age-appropriate for child).	Multiple cysts present but structural integrity preserved, hypertension present, a mild reduction in kidney function (>60-80 ml/min).	Multiple cysts, enlarged kidneys, hypertension present, moderate renal insufficiency (<60 ml/min).
Age	Includes young adults and many children.	Includes young adults and adolescents; occasional children.	Includes primarily individuals older than 30 years of age; some in 20s.
Outcome Measures: **Cyst and kidney volumes**	Progressive increase in cyst and kidney volumes due to addition of new cysts and enlargement of existing cysts. Changes indication of symptoms and eventual loss of renal function.	Growth of existing cysts and addition of new cysts. CRISP study has now defined MR (or CT) as measurements of dynamic changes in cyst and kidney volumes.	Multiple large cysts present. Cyst size/number not likely to be altered by interventions.
GFR	Very slow or no short-term change requiring long follow-up period in majority of subjects; a minority will have rapidly progressive disease; variability and reproducibility of measurements undefined in ADPKD patients with well-preserved kidney function.	Slow rate of progression requiring long follow-up period in large number of subjects; rate of loss of GFR in ADPKD patients with well-preserved kidney function is not well-established. Rate of progression likely to be no greater than 2-3 ml/min/1.73 m^2/year; faster with established hypertension and other poor prognostic factors.	Rate of change and variability of GFR measurements well-established. Existing data suffices for sample size calculations. Rate of progression likely to be 5-6 ml/min/1.73 m^2/year.
Outcome Measure: **Tubular Function**	Markers of tubular injury and function not well-established.	May be more sensitive marker of intervention. Variability and reproducibility of measurements, i.e., maximal urinary concentration, undefined.	Well-established tubular dysfunction; not likely to be reversed by any intervention.
Potential Interventions	Early interventions affecting tubular structure, differentiation, cell proliferation, or apoptosis. Likely will require prolonged follow-up to assess response to therapy.	Anti-fibrotic, anti-hypertensive, anti-secretory, or anti-inflammatory agents. Use of agents such as vasopressin receptor antagonists.	ACE-I; antihypertensive agents. Possibly antisecretory agents.

Chapman et al (FDA) et al. 2007

Challenges for therapy

- Identification of who will progress
- Treatment may be for decades (side-effects, compliance etc)
- Need to act on multiple signaling pathways
- Stage-specific therapies (e.g. anti-proliferative early, V2 antagonists later)

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