Coordinate Expression of the Autosomal Dominant Polycystic Kidney Disease Proteins, Polycystin-2 And Polycystin-1, in Normal and Cystic Tissue

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A second gene for autosomal dominant polycystic kidney disease (ADPKD), *PKD2*, has been recently identified. Using antisera raised to the human *PKD2* protein, polycystin-2, we describe for the first time its distribution in human fetal tissues, as well as its expression in adult kidney and polycystic *PKD2* tissues. Its expression pattern is correlated with that of the *PKD1* protein, polycystin-1. In normal kidney, expression of polycystin-2 strikingly parallels that of polycystin-1, with prominent expression by maturing proximal and distal tubules during development, but with a more pronounced distal pattern in adult life. In nonrenal tissues expression of both polycystin molecules is identical and especially notable in the developing epithelial structures of the pancreas, liver, lung, bowel, brain, reproductive organs, placenta, and thymus. Of interest, nonepithelial cell types such as vascular smooth muscle, skeletal muscle, myocardial cells, and neurons also express both proteins. In *PKD2* cystic kidney and liver, we find polycystin-2 expression in the majority of cysts, although a significant minority are negative, a pattern mirrored by the *PKD1* protein. The continued expression of polycystin-2 in *PKD2* cysts is similar to that seen by polycystin-1 in *PKD1* cysts, but contrasts with the reported absence of polycystin-2 expression in the renal cysts of *Pkd2*^{+/−} mice. These results suggest that if a two-hit mechanism is required for cyst formation in *PKD2* there is a high rate of somatic missense mutation. The coordinate presence or loss of both polycystin molecules in the same cysts supports previous experimental evidence that hetero-

typic interactions may stabilize these proteins. (*Am J Pathol* 1999, 154:1721–1729)

Renal cysts are the primary cause of morbidity in autosomal dominant polycystic kidney disease (ADPKD) but it is evident that the disease phenotype extends beyond the kidney. Cysts are commonly found in the liver and pancreas and have been reported in testis, spleen, ovary, uterus, esophagus, and brain. Moreover, abnormalities suggestive of a generalized disorder of connective tissue, including cardiac valvular abnormalities (especially mitral valve prolapse), intracranial berry aneurysms, colonic diverticulae, inguinal hernia, and a family with Marfanoid habitus, have been described.^{1,2}

Mutations in two genes, *PKD1* and *PKD2*, account for the vast majority of patients with ADPKD. The identification of these genes^{3,4} has thus provided a new opportunity to study the pathophysiology of ADPKD. The predicted proteins appear quite different in structure (the *PKD1* protein, polycystin-1, is ~4 times larger than its counterpart, polycystin-2^{4,5}). Nonetheless, they share a significant region of homology in their transmembrane regions, an area also similar to a family of voltage-gated calcium/sodium channels.^{4,6} Recent evidence indicates that the ADPKD proteins may interact in experimental systems.^{7,8}

PKD2 is less prevalent than *PKD1*, accounting for ~15% of ADPKD cases,^{9,10} but preliminary evidence suggests they share the same spectrum of extrarenal manifestations. In one study, the frequency of hepatic cysts was similar in *PKD1* and *PKD2* patients, although no pancreatic cysts were found in *PKD2* patients;¹⁰ intracranial aneurysms have also been described in *PKD2* families.¹¹ The renal phenotypes are also similar, although *PKD2* patients have milder disease and a lower incidence of hypertension.^{10,12} Consistent with the sug-

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gestion that ADPKD proteins have related functions and similar systemic disease phenotypes, expression of *PKD1*¹³ and *PKD2*⁴ has been found in most human tissues. To date, however, the different cell types expressing these proteins have not been systematically defined.

One area of uncertainty about ADPKD has been understanding the mutational mechanism underlying cyst initiation. Although the germline mutations at *PKD2*¹⁴ and *PKD1*¹⁵ are probably inactivating, controversy exists over the additional steps necessary for focal cyst development. Evidence of loss of heterozygosity in individual PKD1 renal^{16,17} and liver cysts¹⁸ and recent data from targeted disruption of the mouse *Pkd2* gene¹⁹ favor a two-hit mechanism involving somatic inactivation of the normal allele. However, studies of PKD1 cystic tissue have shown polycystin-1 expression^{13,20,21}. No corresponding studies of human PKD2 tissue have yet been described.

To understand the cellular basis for the renal and extrarenal manifestations of ADPKD, we examined the expression of polycystin-2 and polycystin-1 in human fetal tissues at different ages of gestation, using antisera raised to an epitope in the predicted C-terminal region of PKD2 and two previously described polycystin-1 mAbs^{13,21}. The expression of polycystin-2 was also examined in PKD2 cystic kidney and liver tissue for clues to the mutational basis of cyst formation.

Materials and Methods

Production of Antisera to Polycystin-2

A 774-bp segment (2130–2904 nt) of *PKD2* was cloned into the vector pET32a+ (Invitrogen, Carlsbad, CA), in frame with the thioredoxin protein and a His tag. This plasmid was introduced into the *Escherichia coli* strain AD494 DE3 pLysS to synthesize a bacterial fusion protein containing the C-terminal 258 amino acids (aa) of polycystin-2. Following induction with IPTG, recombinant protein was isolated from bacterial lysate by Ni²⁺ affinity chromatography using a 4.6 × 100 mm POROS MC 20 column on a BioCAD workstation (PerSeptive Biosystems, Framingham, MA). Bound protein was eluted on an imidazole gradient and the relevant fractions dialysed against phosphate-buffered saline before immunizing rabbits.

The production and characterization of two polycystin-1 mAbs, PKS-A and 7e12, has been described previously^{13,21}. The epitope detected by PKS-A is contained within the final 233 aa of the C-terminus of polycystin-1, whereas 7e12 was raised to an epitope in the N-terminal region (flank-LRR-flank domain) of polycystin-1.

Generation of a Full-Length PKD2 cDNA Expression Construct (PKD2Pk)

A full-length PKD2 cDNA was produced using the IMAGE clone 239P18 (153473) and a 5' PCR product. The clone begins at the initiation codon and ends at 2970 nt.⁴ The Pk TAG epitope tag recognized by the mAb SV5-Pk

(Serotec, UK)²² was incorporated at the C-terminal end of the protein by replacing the stop codon with the sequence 5'-GACTCGGGAAAGCCGATCCCAAACCCCTT-TGCTGGGATTGGACTCCACCTAGTGA-3'. The PKD2Pk construct was cloned in pCDNA-3 and an internal ribosomal entry site (IRES) from the encephalomyocarditis virus inserted between the cytomegalovirus immediate early promoter and the first in frame ATG.²³ This generates a recombinant protein with an open reading frame of 984 aa and predicted MW of 111 kd.

Plasma Membrane Preparation from Tissues and Cells

Plasma membrane fractions from normal human kidney and COS-1 cells were prepared by established methods^{22,24}. Protease inhibitors (2 mg/ml aprotinin, 1 mmol/L benzamide, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 4 mg/ml pepstatin, 1 mg/ml leupeptin, 1 mg/ml Pefablock; Boehringer Mannheim, Mannheim, Germany) were added to the buffers at all stages of the procedure.

Western Blotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed on 7.5% separating gels using a Bio-Rad Mini Protean II apparatus with molecular weight standards (Rainbow, Amersham Little Chalfont, UK; 14.3–220 kd). Separated proteins were transferred at 30V overnight onto a PVDF membrane (Millipore, Watford, UK). In some experiments, gels were stained with Coomassie, either in parallel to assess migration or after transfer to assess transfer efficiency. Western blotting using enhanced chemiluminescence detection of bound secondary antibody was performed as previously described.¹³

Immunohistochemistry

Fresh tissue was obtained from fetuses (13–40 weeks gestational age), fixed in 10% formal saline, and embedded in paraffin for routine histology. The postmortem findings for each fetus are shown in Table 1 and a summary of the tissues analyzed for each fetus in Table 2. In a small number of cases where single organs were affected, the abnormal tissues were excluded from analysis. Normal adult kidney was obtained from the normal pole of three nephrectomy specimens and processed for histology as for fetal tissues; all three patients (median age, 74 years) underwent nephrectomy because of renal cell carcinoma.

Immunohistochemistry was performed with the polycystin-1 mAbs (PKS-A and 7e12), p30, and preadsorbed p30 (preincubated for 4 hours with a protein G-Pk-recombinant polycystin-2 conjugate), using peroxidase- or fluorescent-conjugated secondary antibodies as previously described.¹³

Table 1. Clinical Details of Fetuses from whom Tissue Was Obtained

Gestational age	Postmortem diagnosis
13 weeks	Normal (spontaneous abortion)
14 weeks	Normal (termination) Skeletal dysplasia (termination) Skeletal dysplasia (spontaneous abortion)
16 weeks	Anencephaly (termination) Anencephaly (termination) Anencephaly (termination) Normal (spontaneous abortion)*
17 weeks	Posterior fossa cyst (termination)
18 weeks	Trisomy 21 (termination) Skeletal dysplasia (termination) Neural tube defect (termination)
20 weeks	Normal (termination) Acute chorio-amnionitis Placental abruption
24 weeks	Prematurity Placental abruption Normal (termination)*
28 weeks	Placental abruption Prematurity
32 weeks	Placental abruption Postoperative death (small bowel atresia)
33 weeks	Antepartum haemorrhage*
36 weeks	Birth asphyxia
39 weeks	Antepartum haemorrhage*
40 weeks	Pulmonary hypoplasia Antepartum haemorrhage

*Only brain tissue was examined.

Analysis of Cystic Tissue

Cystic tissue was obtained from five PKD2 patients (4 kidneys, 3 livers); the germline mutations in four of these patients have been defined (see Table 3). All cysts >2 mm in diameter were counted and graded as positive (+) or negative (−) for polycystin-2 and polycystin-1. A small and variable proportion of cysts, however, showed a heterogeneous pattern of staining with negative areas clearly present in an otherwise positive cell lining or *vice versa*. These were graded as positive/negative (+/−) cysts.

The total number of cysts counted varied between individuals but reflects the maximum in all available cystic tissue.

Materials

Mouse mAbs to the $\alpha 1$ subunit of Na^+/K^+ -ATPase and the epithelial membrane antigen (EMA) were purchased from Upstate Biotechnology (Lake Placid, NY) and Sigma (St. Louis, MO), respectively.

Results

Characterization of the Polycystin-2 Antisera (p30)

The polyclonal antisera, p30, was raised to the C-terminal 258 aa of polycystin-2. This region was selected because it showed no significant sequence similarity to polycystin-1 or any other known proteins. Recently a polycystin-2-like protein, PKDL, has been described but cross-reactivity with this molecule is unlikely because the antigenic region shows only a low level of identity (26%) and because PKDL appears to have a restricted tissue distribution.^{25,26}

The specificity of p30 was initially tested by Western blotting and dual immunofluorescent staining of COS-1 cells transiently transfected with the Pk epitope tagged PKD2 cDNA expression plasmid (PKD2Pk). Western blot analysis indicated high levels of a 110-kd protein in PKD2Pk transfected COS-1 cells with the p30 and Pk antibodies (Figure 1A). In addition, p30 detected the endogenous PKD2 protein as a weaker but similarly sized band in untransfected COS-1 cells (Figure 1A). Dual immunofluorescent labeling of PKD2Pk transfectants also showed that only cells labeling with the Pk mAb were strongly detected with p30, showing its specificity for the PKD2 protein (data not shown).

The specificity of p30 for endogenous polycystin-2 was further tested by Western analysis in plasma membrane fractions (enriched for the membrane protein, Na^+/K^+ -

Table 2. Cellular Expression of Polycystin-2 and Polycystin-1 in Human Fetal Tissues

Tissue	Cellular expression
Kidney	Early nephron precursors (C and S bodies, ureteric bud show weak expression); proximal tubular expression remains prominent up to 28 weeks gestation, distal tubule and collecting duct expression persists to term
Pancreas	Ductal epithelial cells (small and large ducts), acinar tissue, and islet cells
Liver	Biliary epithelium (small and large ducts), hepatocytes, and nucleated red cells
Lung	Cuboidal epithelium lining terminal sacs (weak at 13 weeks but negative at 20 weeks), respiratory epithelium lining airways, chondrocytes, and smooth muscle cells
Brain	Ependymal lining of cerebral ventricles, neuronal cell bodies (positive from 33 weeks in cerebral cortex, pons, and medulla), endothelial and smooth muscle cells of arteries
Bowel	Squamous epithelium of esophagus, small bowel enterocytes, colonic epithelium, longitudinal and circular smooth muscle layers, neuronal cells of myenteric plexus
Heart and vessels	Myocardial and endocardial cells, smooth muscle cells, and endothelial cells in vessels from different tissues
Reproductive organs	Ductal lining epithelium of epididymis, vas deferens, and fallopian tubes, oocytes, Leydig cells
Placenta	Trophoblasts, amnion lining cells, smooth muscle and endothelium of umbilical artery and vein
Other tissues	Skeletal muscle cells, fetal and adult cortex (adrenal), Hassell's corpuscles (thymus), nucleated red cells (spleen)

Table 3. Polycystin-2 Immunoreactivity in PKD2 Cystic Kidney and Liver Tissue

Patient	Mutation	Predicted effect on protein	References	Tissue analyzed	Total cysts	No. pos. cysts (%)	No. neg. cysts (%)	No. pos./neg. cysts (%)
OX964	not detected	?		Kidney	82	44	31	7
TOR-PKD6	IVS5+1G→A	In-frame deletion of 360–440aa	Pei* (unpublished results)	Kidney	23	12	8	3
				Liver	1	1	0	0
TOR-PKD8	2159insA	Truncation after 720aa	34	Liver	9	7	0	2
TOR-PKD39	124ins52	Truncation after 41aa	Pei (unpublished results)	Kidney	19	8	10	1
				Liver	13	12	1	0
JR111:3	PKD2 deletion	Loss of polycystin-2	35	Kidney	66	26	25	15
Totals					213	110 (51.6)	75 (35.2)	28 (13.2)

*Pei, Y.

ATPase) prepared from 3 human adult kidneys. A major ~110-kd band was detected in the 3 kidney samples; no signal was detected with preimmune sera (Figure 1B). A very weak band (<110 kd) was also detected by p30 in both human kidney membrane fractions and COS-1 transfectants and a faint higher molecular weight band of >220 kd was sometimes detectable on longer exposures. The presence of these minor species in the transfected cells (Figure 1A) suggests that they are forms of the polycystin-2 protein, possibly a splice variant or degradation product (<110 kd) or multimer/aggregate (>220 kd). All three bands disappear when p30 is preincubated with recombinant polycystin-2, further indicating that the antisera is polycystin-2-specific (data not shown). Taken together, these results indicate strong specificity of p30 for both recombinant and endogenous polycystin-2.

Expression of Polycystin-2 and Polycystin-1 in Normal Human Adult Kidney

Initial studies on adult human kidney with p30 showed polycystin-2 to be expressed mainly in the medullary collecting ducts, cortical collecting ducts, and distal convoluted tubules (Figure 2A) with no detectable signal with preimmune sera from the same rabbit (Figure 2B). Similarly, no detectable signal was seen in human kidney when p30 was preincubated with the recombinant full-length polycystin-2 protein (data not shown).

Although our initial description of polycystin-1 expression with the PKS-A mAb¹³ indicated a more uniform tubular expression in the adult kidney, re-analysis has suggested a more pronounced distal pattern identical to

Figure 1. Western blotting of (A) total cell lysates from control (C; 20 μ g) or PKD2Pk transfected (T; 10 μ g) COS-1 cells with preimmune sera, the PKD2 antisera, p30, or the mAb to the Pk tag. A 110-kd protein is detected by p30 in control (endogenous PKD2) and transfected COS-1 cells (recombinant plus endogenous PKD2), whereas the Pk mAb detects only recombinant polycystin-2. B: Plasma membrane enriched fractions from three normal human kidneys (NK1–3; 50 μ g) revealed a major 110-kd band when detected with p30 but not with preimmune sera. Redetection of the preimmune filter with a mAb to the α 1 subunit of Na⁺-K⁺-ATPase showed clear evidence of the expected 100-kd protein (data not shown).

Figure 2. Staining of the cortex of adult human kidney with (A) the PKD2 antisera p30, (B) preimmune sera for p30, and (C) polycystin-1 antibody, PKS-A. No signal is seen with the preimmune sera, whereas antibodies to both ADPKD protein show prominent staining in distal convoluted tubules (arrows) and weaker staining of proximal tubules. Glomeruli (g) are negative. D-F: Dual fluorescent labeling of adult human kidney shows colocalization of polycystin-2 (D) and polycystin-1 (E) in the medullary collecting ducts. F represents the merged color image of D and E. A nuclear counterstain (DAPI) is displayed in F. Magnification, X200.

that of polycystin-2 (Figure 2C). In both cases weaker proximal tubular expression was also seen (Figure 2, A and C). Colocalization of the two polycystin proteins could be demonstrated clearly by dual color immunofluorescence (Figure 2, D-F) in medullary collecting ducts, which strongly express both proteins.

Expression of Polycystin-2 and Polycystin-1 in Human Fetal Kidney and Other Fetal Tissues

Further studies were conducted on fetal tissues of different ages (13–40 weeks) to assess the expression pattern

of both ADPKD proteins in the developing kidney and other organs. In each case the pattern of expression was identical for the polycystin-1 antibodies (PKS-A, 7e12) and the PKD2 antibody (p30) (see Figure 3, B-D, and Figure 4, A-C). To illustrate as many fetal ages and tissues as possible, results of polycystin-2 expression are mainly illustrated (Figures 3 and 4).

As previously demonstrated for polycystin-1,¹³ polycystin-2 expression was found in all nephron segments in the developing kidney (Figure 3). Faint but detectable expression was seen in the earliest epithelial precursors (S and comma-shaped bodies and proximal and distal

Figure 3. Polycystin-2 (A, B, E-H) and polycystin-1 (C, D) expression in human fetal kidney at different stages of development. A-D: At 14 weeks gestation, prominent expression by maturing tubules (t) in the nephrogenic zone (with weak expression by earlier nephron precursors), comma-shaped (c) and S-shaped (s) bodies, and the proximal and distal branches of the ureteric bud (u) was observed. B-D: Note the apical and basal expression (arrows) evident in some early collecting ducts. On serial sections, an identical expression pattern (arrows) can be seen for polycystin-2 (B) and polycystin-1, PKS-A (C), and 7e12 (D) in the medullary collecting ducts. E: At 17 weeks gestation, the apical and basal expression pattern (arrows) persists in some medullary collecting ducts. F: At 20 weeks gestation, expression by proximal (p) and distal convoluted tubules (dct) is equally strong and also detectable in the parietal epithelium of Bowman's capsule (arrow). G: At 36 weeks gestation, toward term, expression remains strong in the medullary collecting ducts. H: At 40 weeks gestation, the cortical collecting ducts (cd) and distal convoluted tubules (dct) retain a higher level of expression than the proximal tubules at term, reflecting the pattern seen in the adult kidney. Glomerular tufts (g) were essentially negative though Bowman's capsule (arrow) was positive especially at points of tubularization. Magnifications, X200 (A, D, E, F, I) and X400 (B, C, G, H).

Figure 4. Polycystin-2 (A, D-L) and polycystin-1 (B, C) expression in nonrenal tissues during development. A-C: At 14 weeks, liver shows positive expression by a bile duct (b) as well as by hepatocytes (h) and nucleated red cell precursors. Serial sections show that polycystin-2 expression (p30; A) is identical to that of polycystin-1 (PKS-A B, 7e12 C). D: At 20 weeks, pancreas shows expression by the epithelium of early branching pancreatic ducts (d); acinar tissue (a) was also positive. E: At 20 weeks, lung shows prominent expression by the stratified columnar epithelium (e) of the developing trachea. Note also weaker expression by chondrocytes (c) and smooth muscle cells. F: At 24 weeks, brain (pons) shows positive expression by the ependymal lining (arrows) of the fourth ventricle. G: At 24 weeks, brain shows positive expression in the smooth muscle (m) and endothelium (arrows) of a medium-sized intracranial artery. H: A 39 weeks, brain (cerebral cortex) shows moderate expression in neuronal cell bodies. I: At 36 weeks, ileum shows expression by enterocytes (e). Expression was detectable also in the myocytes of the circular and longitudinal muscle layers (not shown). J: At 36 weeks, heart sectioned at the level of the papillary muscles shows expression by myocardial (m) and endocardial (arrows) cells. K: At 36 weeks, epididymis shows expression by the lining ductal epithelium. L: At 36 weeks, testis shows prominent expression by Leydig cells (l) with weaker expression by developing Sertoli cells (s). Magnification, $\times 400$.

branches of the ureteric bud) but expression was most marked in the maturing proximal and distal tubules, collecting ducts, and transitional epithelium. Striking apical and basal surface membrane expression was observed in some early medullary collecting ducts (Figure 3, B-E).

Proximal tubular expression was prominent up to 28 weeks but appeared to decline in intensity thereafter. In contrast, medullary collecting duct (Figure 3G) and cortical collecting duct (Figure 3H) expression was maintained right up to 40 weeks.

Figure 5. Sequential sections of PKD2 cystic liver from patient TOR-PKD39 (A-C) and PKD2 cystic kidney, OX964 (D-F), stained for polycystin-2 (A, D), polycystin-1 (B, E), and the distal tubular marker EMA (C, F). Note the positive expression of all three proteins in the epithelial lining of a large cyst (c) (A-C). D: An example of a cyst with a hyperproliferative cyst lining that is negative for polycystin-2 (arrows). Interestingly, this cyst is also negative for polycystin-1 expression (E), although EMA expression is present (F). A tubule (t) positive for all three proteins can be seen nearby. Magnification, $\times 400$.

The results for the other tissues are summarized in Table 2 and examples of different nonrenal cell types expressing polycystin-2 are shown in Figure 4.

Expression of Polycystin-2 and Polycystin-1 in PKD2 Kidney and Liver

Cystic tissue was analyzed for polycystin-2 expression from five different PKD2 patients (see Table 3 for details). In two cases (TOR-PKD39 and JR111:3) the germline mutations are predicted to remove the area used to raise the p30 antibody (see Table 3 for details), which consequently will recognize only the protein encoded by the normal allele. In the other cases the mutation is not known (OX964), is predicted to generate an in-frame change preserving the p30 epitope (TOR-PKD6), or is a frame-shifting change 9 aa into the region used to generate the antibody (TOR-PKD8) and hence, the mutant protein is probably not recognized by p30.

Overall, the analysis shows that 64.8% of cysts stained with p30, although only partial staining was seen in 13.2%, and a significant level of negative cysts (35.2%) was observed. The number of negative cysts was lower in cystic liver than in cystic kidney. The reason for this is unclear, although the total number of liver cysts available for analysis was much lower as a proportion of the total number of cysts analyzed (Table 3). In the two patients in which only the normal protein was detectable, similar figures were obtained with 63.3% of cysts positive (16.3% staining partially); we did not observe a similar pattern of heterogeneous staining in non-cystic tubular epithelium. Analysis of serial sections for polycystin-1 immunoreactivity showed a striking coordinance, with each polycystin-2-negative cyst also lacking polycystin-1. Examples of cysts either positive or negative for polycystin-2 and polycystin-1 are illustrated in Figure 5.

Polycystin-2 expression was also analyzed in 3 PKD1 kidneys. This showed that the majority of cysts (51/70) were positive (one partially) and, again, coordinate expression with polycystin-1 was noted in each cyst.

Discussion

Our studies of expression of polycystin-2 and polycystin-1 in normal fetal tissues and adult kidney have shown a strikingly similar pattern of expression. These results of colocalization and codevelopmental expression are consistent with experimental results indicating an interaction between these proteins and support the view that they may form parts of the same complex or pathway.^{7,8} A related role for the two polycystin proteins is also consistent with the clinical similarities of PKD1 and PKD2.

In fetal and adult kidney, the expression pattern of polycystin-2 was identical to that observed for polycystin-1 and similar to a consensus that is emerging for polycystin-1. This consists of expression early in nephrogenesis, which is most marked in the maturing proximal

and distal tubules and collecting ducts but more pronounced in the distal tubules and collecting ducts by 40 weeks. Apart from the study of Griffin et al²⁷ that observed no polycystin-1 expression in the adult, this later distal expression pattern persisting into adult life has been observed in several other studies.^{20,28,29} The expression of the ADPKD proteins appears only weakly in the earliest nephrogenic precursors, and is consistent with data from the *Pkd1*^{-/-} knockout mouse,³⁰ where formation of the nephron appears to occur normally; the role of these proteins is thus probably in tubular elongation and the maintenance of tubular architecture rather than in epithelial induction.

The subcellular localization of the ADPKD proteins remains uncertain with evidence for polycystin-1 expression on the apical and/or basolateral plasma membranes^{20,31} or a predominant intracytoplasmic location.^{13,27} We observed clear plasma membrane (basal and apical) expression of polycystin-2 (and polycystin-1) in developing medullary collecting ducts at 14–17 weeks, although in other tubules the expression appeared to be intracytoplasmic. Western blot analysis showed membrane associated protein in this study and elsewhere,²⁰ although it is not clear what proportion is located on the plasma membrane. It is possible that there is continuous recycling of both proteins with appropriate membrane targeting dependent on prior interactions with other members of a polycystin complex or on specific cell-cell or cell-matrix interactions.

In other organs commonly affected by cysts, such as the liver and pancreas, polycystin-2 expression mirrors that of polycystin-1^{20,27} with protein detected in the ductal epithelial structures of both organs persisting up to 40 weeks of gestational age. Significantly, prominent expression of both polycystin molecules was also found in all other epithelial tissues including the lung, small and large bowel, brain, reproductive organs, placenta, and thymus, indicating a more general role for these proteins in the maintenance of epithelial differentiation and organization. Furthermore, the expression of both proteins by other cell types such as muscle, endothelial, and neuronal cells indicate a more widespread role in the formation and/or maintenance of nonepithelial tissues. This would certainly be consistent with the many systemic non-cystic features described in ADPKD. The function(s) that the ADPKD proteins may have in these diverse cell types can as yet only be guessed at, with structural predictions suggesting a role in signaling, triggered by cell-cell/matrix interactions⁵ or a possible involvement in ion transport.^{4,6,32}

In general, our results in nonrenal fetal tissues are consistent with those previously described for polycystin-1²⁷ but extend them by showing coexpression of the polycystin molecules by a wide variety of different cell types. Nevertheless, some questions remain. We previously described the highest levels of adult *PKD1* gene expression in the brain.¹³ Unlike others,²⁹ we did not observe polycystin-1 (or polycystin-2) expression by astrocytes, but rather by neuronal cell bodies throughout the developing brain. However, no neural phenotype has been reported for ADPKD, indicating that the presence of

one mutant allele does not disrupt neural development. It will be interesting to see if there is a neural phenotype in a fuller description of the *Pkd1*^{-/-} and *Pkd2*^{-/-} knockout mice.^{19,30} One clear phenotype affecting the brain and associated with ADPKD, is an increased level of intracranial aneurysms. Interestingly, we found staining for both ADPKD proteins in the endothelial and smooth muscle cells of the arteries in the brain and other organs. Unlike the study of Griffin et al,³³ however, staining of muscle cells was evident without the requirement of protease treatment. This pattern of staining suggests a direct role for the polycystin molecules in the pathology of this disease complication.

Our studies of PKD2 cystic tissue showed that the majority of cysts stained for polycystin-2. In the 2 cases with defined germline mutations that eliminate the p30 epitope, this must represent protein encoded by the normal allele. A similar pattern where the majority of cysts stain has been found in analysis of PKD1 cystic tissue with polycystin-1 antibodies,^{20,27,28,31} including cases with defined mutations that remove the antibody epitope^{13,21}. The PKD2 results, however, differ from those found in mice heterozygous for a *Pkd2* null mutation, where no polycystin-2 expression was detected in cysts, suggesting that somatic loss of the normal product had occurred.¹⁹ However, these animals had relatively few cysts and it will be interesting to see if any show polycystin-2 expression in a more detailed analysis. Our results could be consistent with a two-hit hypothesis if the staining is of protein inactivated by a missense change. The variable number of positive cysts between individual tissues suggests that this is a possibility because the nature of the somatic mutation is likely to be a chance event. Alternatively, these results may indicate that cyst initiation can occur without loss of the normal protein by a dosage effect or possibly due to somatic changes at other loci (such as *PKD1*¹⁶), which may encode proteins that interact with the polycystin complex. Analysis of individual PKD2 cysts for somatic mutations would help resolve whether a second genetic event at this locus is always required for cyst development.

Analysis of PKD2 cystic tissue with a polycystin-1 antibody also showed staining of most cysts. Interestingly, cysts negative for polycystin-2 were also negative for polycystin-1 (Figure 5); a similar concordant expression pattern was found in PKD1 cysts. Although the loss of expression of one polycystin molecule may be explained by a germline and a somatic mutation, it is unlikely that somatic genetic events could account for the removal of the second ADPKD protein in the same cyst. More credible, and consistent with the polycystin molecules forming a multimeric complex, absence of one may lead to rapid degradation of other binding partner(s). Experimental evidence of polycystin-2 stabilizing polycystin-1 has been described.⁸

Note added in proof: Recent evidence of somatic mutations in a proportion of PKD2 renal cysts has been described.^{35,36}

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