Loss of the Polycystic Kidney Disease (*PKD1*) Region of Chromosome 16p13 in Renal Cyst Cells Supports a Loss-of-Function Model for Cyst Pathogenesis

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Abstract

It is not known whether mutations in the *PKD1* gene cause autosomal dominant polycystic kidney disease (PKD) by an activating (gain-of-function) or an inactivating (loss-of-function) model. We analyzed DNA from cyst epithelial cells for loss of heterozygosity (LOH) in the *PKD1* region of chromosome 16p13 using microsatellite markers. 29 cysts from four patients were studied. Five cysts from three patients had chromosome 16p13 LOH. Four of the cysts had loss of two chromosome 16p13 markers that flank the *PKD1* gene. In two patients, microsatellite analysis of family members was consistent with loss of the wild-type copy of *PKD1* in the cysts. In the third patient, 16p13 LOH was detected in three separate cysts, all of which showed loss of the same alleles. Chromosome 3p21 LOH was detected in one cyst. No LOH was detected in four other genomic regions. These results demonstrate that some renal cyst epithelial cells exhibit clonal chromosomal abnormalities with loss of the wild-type copy of *PKD1*. This supports a loss-of-function model for autosomal dominant PKD, with a germline mutation inactivating one copy of *PKD1* and somatic mutation or deletion inactivating the remaining wild-type copy. (*J. Clin. Invest.* 1997. 99:194–199.) Key words: chromosomes, human, pair 16 • gene deletion • chromosome deletion • kidney, polycystic, autosomal dominant • tuberous sclerosis

Introduction

Autosomal dominant polycystic kidney disease (ADPKD)[1] is one of the most common hereditary disorders in the general population, accounting for 8 to 10% of all cases of end-stage renal disease (1). 85% of ADPKD is linked to the *PKD1* gene on chromosome 16p13 (2), with most of the remaining cases linked to PKD2 on chromosome 4q13-23 (3).

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1. *Abbreviations used in this paper:* ADPKD, autosomal dominant polycystic kidney disease; LOH, loss of heterozygosity.

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The *PKD1* gene was identified in 1994 (4). Polycystin, the protein product of the *PKD1* gene, is predicted to be a very large (4,302 amino acids) membrane-associated glycoprotein (5–7). The PKD2 gene, identified in 1996, is predicted to encode a 968 amino acid integral membrane protein (3).

Although a great deal is known about the biology and biochemistry of renal cysts (8–16), it is not known how *PKD1* mutations cause cysts. *PKD1* germline mutations could activate polycystin, giving it a new or unregulated function. Alternatively, *PKD1* germline mutations could cause loss of polycystin function, with somatic inactivation of the remaining wild-type copy required for cyst development (8, 9).

If the inactivation or loss-of-function model is correct, loss of wild-type *PKD1* could occur through intragenic mutation or by deletion of the region of chromosome 16p13 containing the wild-type gene. Large chromosomal deletions in clonal cell populations can be detected using a loss of heterozygosity (LOH) analysis. Loss of heterozygosity refers to a DNA marker with a one-allele (homozygous) pattern in DNA from a lesion or tumor when compared with a two-allele (heterozygous) pattern in the patient's normal DNA (10).

We analyzed ADPKD cyst cells for LOH in the chromosome 16p13 region to look for chromosomal deletions that inactivate one copy of *PKD1*.

Methods

Patients and specimens. Four patients whose end-stage polycystic kidneys required surgical resection were studied. Patients 168, 233, and 311 have family histories consistent with ADPKD. Patient 305 was adopted and has no children. The kidneys were shipped in sterile bags on ice by overnight delivery. This study was approved by the Institutional Review Board of the Brigham and Women's Hospital.

Preparation of cyst cells. For all cyst preparations, the domes of single cysts were dissected from surrounding tissue, removed, and rinsed in PBS. To analyze cells from individual cysts, cysts were chosen that could be separated from neighboring cysts. Large cysts that appeared to represent the confluence of smaller cysts were avoided.

To isolate cyst epithelial cells directly from the kidneys, without culturing, the interior surface of the dome of individual cysts from kidneys 305 and 311 was scraped with a razor blade, leaving the cyst wall intact. DNA was prepared from cells that separated from the cyst wall with scraping. Adherent clumps of cells from the cyst wall were excluded from the preparations.

Cyst epithelial cells were cultured from kidneys 168, 233, and 311 (11). For cell culture, the cyst dome was minced with a razor blade and incubated for 16 h at 37°C in 0.2% type I collagenase (Sigma Chemical Co., St. Louis, MO) in F12 media. After the collagenase treatment, the tissue was washed five times in PBS, and plated directly into individual wells of a 24-well culture dish with 50% Hamm's F12/50% DME media supplemented with 5% FCS, penicil-

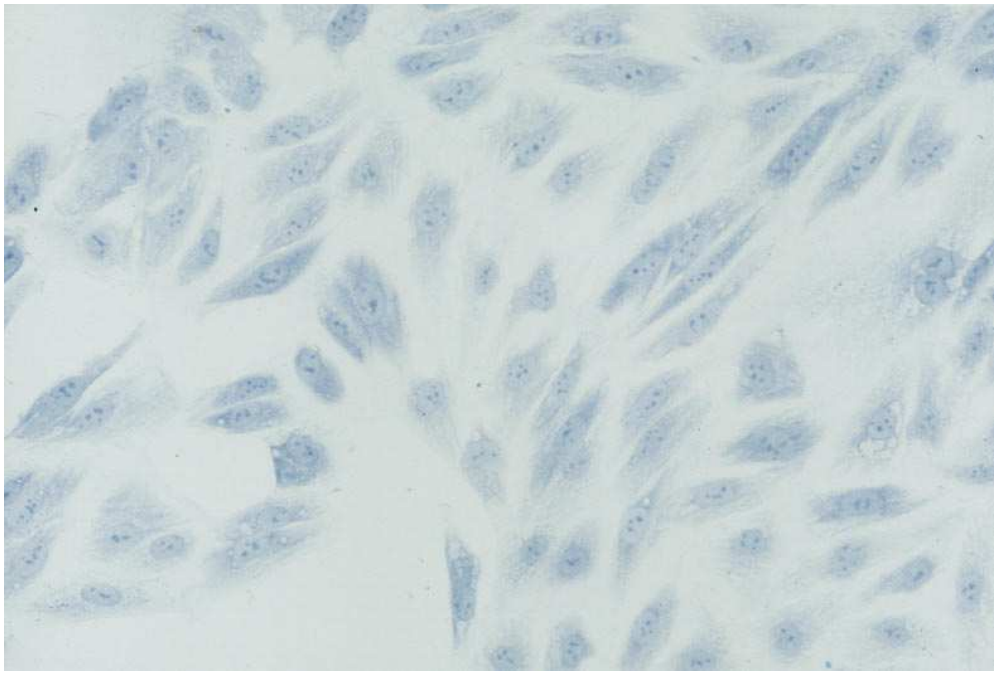

Figure 1. Wright-Giemsa–stained cultured cyst cells from patient 168.

lin, streptomycin, and amphotericin B. The next day, debris was removed from adherent cells by a wash in PBS followed by refeeding with the F12/DME/FCS media. 18 h after plating, cultures contained 8–12 separate clusters of cells that grew as homogeneous monolayers (Fig. 1) and could be split at a 1:3 ratio for three or four passages. The number of cells that morphologically resembled fibroblasts varied among different cultures from about 1% to 10%. Cells were harvested for DNA analysis at the initial passage.

Loss of heterozygosity analysis. DNA for PCR analysis was prepared from the renal cyst cells and from the renal artery and renal vein (the source of normal DNA) of each patient by overnight incubation at 50°C in 50 mM KCl, 10 mM Tris, pH 8.3, 1.5 mM $MgCl_2$, 100 μg/ml bovine serum albumin, and 100 μg/ml proteinase K. The proteinase K was inactivated by a 10-min incubation at 95°C.

PCR amplification of the DNA was performed using short tandem repeat markers from chromosome 16p13 (Fig. 2): D16S283, Kg8, and D16S525 (12, 13); markers from other chromosomal regions including D4S423 and D4S1563 (14) from the PKD2 region of chromosome 4; D3S1478 (chromosome 3p21); IFN (chromosome 9p); p53 (chromosome 17p13); and D9S66 (chromosome 9q34).

1μl of the DNA preparations, containing ~ 100 ng of DNA, was used in a 10-μl PCR reaction. Most markers were amplified for 30 cycles using a 55°C annealing temperature. PCR was performed with [^{32}P]dGTP in the reaction mix (15, 16). PCR products were analyzed by denaturing 8 M urea polyacrylamide gel electrophoresis followed by autoradiography. LOH was determined by visual comparison of autoradiographic signals obtained from amplification of cyst and control DNA. Examples of typical LOH results at the loci D16S525, Kg8, and D16S283 are shown in Figs. 3, 4, and 5. All PCR reactions showing LOH were repeated at least three times for confirmation.

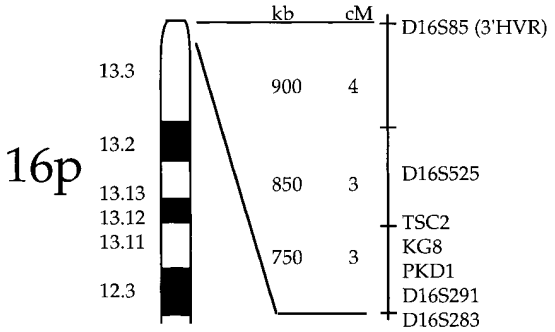

Figure 2. Map of chromosome band 16p13. The relative positions of the markers used in this study and the *PKD1* and *TSC2* genes (4, 12) are shown. Distance in centimorgans (cM) and kilobases (kb) is indicated.

Results

Chromosome 16p13 loss of heterozygosity in cyst cells. DNA from 29 individual renal cysts was examined for loss of heterozygosity using three microsatellite markers in the *PKD1* region of chromosome 16p13 (Fig. 2). In preliminary studies, we

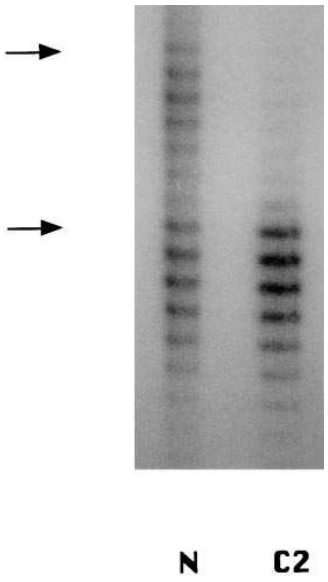

Figure 3. Loss of chromosome 16p13 in cyst 2 from patient 305. DNA was analyzed with the chromosome 16p13 marker D16S525. Each allele has a multiple band pattern. The upper band of each allele is indicated with an arrow. The upper allele is lost in cyst 2 (*C2*). N, normal DNA.

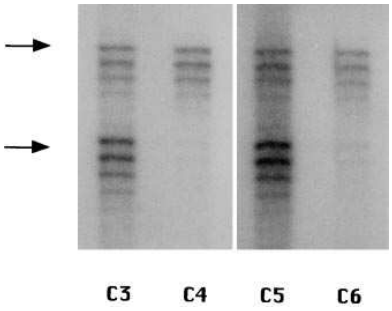

Figure 4. Loss of chromosome 16p13 in cysts from patient 311. Normal DNA and cyst DNA (*C*) were analyzed with the marker D16S525. The upper band of each allele is indicated with an arrow. The lower D16S525 allele is lost in C4 and C6.

Table I. Detection of LOH in ADPKD Renal Cysts

Kidney	Method	No. cysts analyzed	No. cysts with 16p13 LOH	No. cysts with 3p LOH
168	Culture	3	1	0
233	Culture	8	0	0
305	Scraped	5	1	0
311	Culture	3	0	0
	Scraped	10	3	1

did not detect LOH in any cysts when the total cyst wall was used for DNA preparation. We therefore isolated epithelial cells from individual cysts using one of two techniques: either the epithelial cells were scraped from the interior surface of the cysts using a razor blade, or the epithelial cells were grown in culture.

Chromosome 16p13 LOH was detected in a total of five cysts (Table I): one cultured cyst from patient 168 (Fig. 5); one scraped cyst from patient 305 (Fig. 3); and three scraped cysts from patient 311 (Fig. 4). Each of these LOH results was reproducible in at least three separate PCR reactions. No LOH was detected in cysts from patient 233. Patients 168 and 311 were informative at two chromosome 16p13 markers. In the cyst from patient 168, LOH was detected at both D16S283 and Kg8 (Table II, and Fig. 5, *A* and *B*). These markers flank the *PKD1* gene (Fig. 2). In all three cysts from patient 311, LOH was detected at two markers (D16S283 and D16S525) that flank the *PKD1* gene (Table II).

In the loss-of-function model, each time LOH is detected in a cyst from one patient, the same chromosome 16p13 alleles (i.e., the alleles on the copy of chromosome 16p13 containing wild-type *PKD1*) should be lost. Patient 311 had 16p13 LOH in three separate cysts. Consistent with the model, the same alleles of D16S525 and D16S283 were lost from each of the three cysts (Fig. 4).

Loss of wild-type PKD1. We analyzed DNA from relatives of patient 168 to determine whether the lost copy of chromosome 16p13 contained the mutant or the wild-type *PKD1*. As shown in Fig. 5 *A*, the upper Kg8 allele is present in all three affected individuals (patient 168, her daughter, and her niece). Therefore the upper allele is predicted to be on the copy of chromosome 16p13 carrying the germline *PKD1* mutation. Consistent with the loss-of-function model, the lower Kg8 allele (predicted to be on the copy of chromosome 16p13 containing the wild-type *PKD1*) is lost in cyst 2. Analysis of the family members with D16S283 was also consistent with loss of the wild-type *PKD1* in cyst 2. Patient 168, her daughter, and her niece all have the lower D16S283 allele. The upper D16S283 allele, which is therefore predicted to represent the wild-type copy of *PKD1*, is lost in cyst 2.

DNA was also analyzed from the affected son of patient 305. At the marker D16S525, the son is a homozygote for the lower

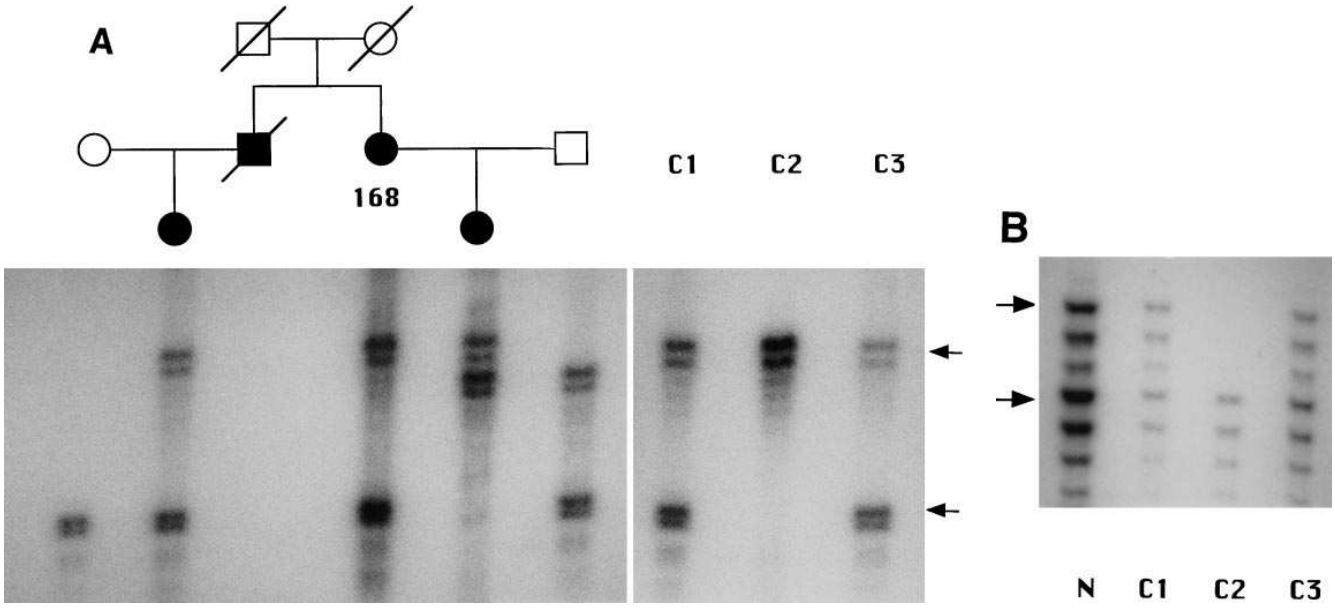

Figure 5. Loss of wild-type *PKD1* in cyst 2 from patient 168. DNA from patient 168 and her family were analyzed with the chromosome 16p13 marker Kg8 (*A*). Each allele has a two-band pattern. Arrows indicate the position of the upper band of the two alleles in DNA from patient 168. Her affected daughter and niece have inherited the upper Kg8 allele. Both alleles are present in cysts 1 and 3 (*C1*, *C3*) from patient 168. Loss of the lower allele is seen in cyst 2 (*C2*). (*B*) DNA from patient 168 (*N*) and the three cysts were analyzed with the chromosome 16p13 marker D16S283. Each allele has a multiple band pattern. The upper band of each allele is indicated with an arrow. Loss of the upper allele is seen in cyst 2 (*C2*).

Table II. Pattern of Allelic Loss in Six Renal Cysts

Patient	Cyst number	D16S283 16p13	Kg8 16p13	D16S525 16p13	D3S1478 3p21
168	C2	LOH	LOH	1	2
305	C2	1	1	LOH	2
311	C1	LOH	1	LOH	2
	C4	LOH	1	LOH	2
	C6	LOH	1	LOH	2
	C10	2	1	2	LOH

LOH, loss of heterozygosity was detected at the marker; 1, the marker is homozygous in that patient; and 2, the marker is heterozygous and LOH was not detected.

allele seen in Fig. 3. Therefore, the upper allele in patient 305 would be predicted to be on the wild-type copy of chromosome 16p13. Loss of the upper allele is seen in Fig. 3. Patient 311 is adopted and DNA from his family members was not available.

Chromosome 3p loss of heterozygosity. To assess the specificity of the chromosome 16p13 allele loss, the cysts were analyzed with markers from four other genomic regions that were not suspected to play a role in cyst development but that are frequently lost in other human tumors: p53 (chromosome 17p13), IFN (chromosome 9p), D9S66 (chromosome 9q34), and D3S1478 (chromosome 3p21). Patient 168 was informative at all regions except 9p; patients 305 and 311 were informative at all of the control genomic regions. We also looked for LOH in the *PKD2* region of chromosome 4 in all four patients using the markers D4S423 and D4S1563.

LOH was found only once using these additional markers: LOH at D3S1478 was detected in one cyst from patient 311 (Fig. 6). This cyst did not have chromosome 16p13 LOH (Table II). No LOH was detected on chromosome 4q or at any of the other chromosomal regions. These results were analyzed for statistical significance using the McNemar test. Chromosome 16p13 LOH occurred more frequently than chromosome 17p13 LOH ($P = 0.025$), chromosome 9p LOH ($P = 0.05$), chromosome 9q34 LOH ($P = 0.025$), chromosome 4q LOH ($P = 0.025$), or chromosome 3p LOH ($P = 0.10$).

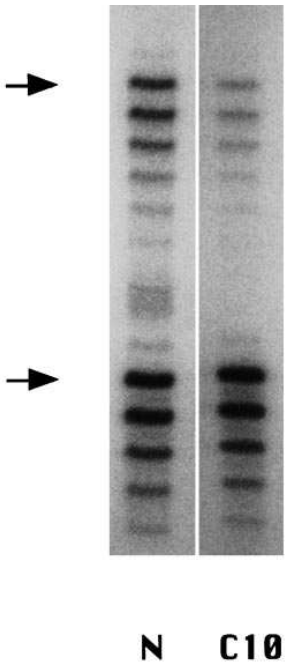

Figure 6. Loss of chromosome 3p21 in a cyst from patient 311. DNA was analyzed with D3S1478. Each allele is represented by a multiple-band pattern, the upper allele of which is indicated with an arrow. Both alleles are present in normal DNA. Loss of the upper allele is seen in Cyst 10 (*C10*).

Discussion

We developed two methods for isolating epithelial cells from renal cysts, and found loss of the *PKD1* region of chromosome 16p13 in epithelial cell preparations from patients with ADPKD. Five cysts from three patients had 16p13 LOH. 21 cysts were analyzed from these three patients (Table I), giving a frequency of LOH of 24%. In one patient, three separate cysts showed chromosome 16p13 LOH, each with loss of the same alleles. In the other two patients, analysis of family members demonstrated that the lost *PKD1* is predicted to be the wild-type copy. These findings are consistent with a loss-of-function model of cyst pathogenesis.

The loss-of-function model predicts that wild-type *PKD1* is inactivated in every cyst. However, chromosome 16p13 LOH was detected in only 5 of 21 cysts (24%) from three patients. There are at least three possible reasons why LOH was not seen at a higher frequency. First, an LOH analysis detects large regions of chromosomal loss. The lack of LOH in some cysts may indicate that the spectrum of inactivating somatic mutations includes small deletions, point mutations, and/or methylation changes. We did not look for point mutations or small deletions in the wild-type copy of *PKD1.*

Second, some of the cyst epithelial cell preparations could have contained nonepithelial cells, such as inflammatory cells or fibroblasts. This is particularly likely with the cyst cells that were scraped from the interior cyst surface. LOH would not be detected using a PCR-based method if a significant number of nonepithelial cells were mixed with the epithelial cells.

A third possible explanation for the relatively low frequency of LOH in ADPKD cysts is that inactivation of wild-type *PKD1* "unmasks" a gain-of-function germline *PKD1* mutation. In this case, loss of wild-type *PKD1* would potentiate, but not be required for, cyst growth. The relative intensities of the two alleles in uncultured cysts with LOH (Figs. 3 and 4) suggest that most cells have lost one copy of *PKD1.* Therefore, even if the loss of *PKD1* is not required for the initiation of cyst growth, when it occurs it must be an early event.

The LOH detected in this study was specific to chromosome 16p13, with the exception of one cyst in which chromosome 3p LOH was detected. Chromosome 16p13 LOH occurred more frequently than LOH at p53 ($P = 0.025$), chromosome 9p ($P = 0.05$), chromosome 9q34 ($P = 0.025$), chromosome 4q ($P = 0.025$), or chromosome 3p ($P = 0.025$). Chromosome 16p LOH is infrequent in other human tumors, with the exception of renal angiomyolipomas from patients with tuberous sclerosis (15). In a study of 33 renal carcinomas, none had 16p13 LOH despite a mean number of LOH regions per tumor of 2.5 (17). The significance of the chromosome 3p LOH is unclear. It could indicate a multi-step model (18) of cyst pathogenesis, with inactivating mutations in other genes occurring during cyst growth. The chromosome 3p LOH (Fig. 6) was not as complete as the chromosome 16p13 LOH in other cysts from the same patient (Fig. 4), suggesting that the chromosome 3 LOH may be a later event affecting only a fraction of the cyst epithelial cells.

Our data support a loss-of-function model for cyst development in ADPKD. Consistent with this model is the fact that the 14 *PKD1* germline mutations that have been identified so far are potentially inactivating (4, 19). The mutations include a translocation in which the entire 3′ half of the protein is lost (4), intragenic deletions (4, 19), aberrant splicing mutations (20), and nonsense mutations (19, 21, 22). No mutation has been detected in more than one family.

The high frequency of somatic mutation in normal human kidney epithelial cells (23) supports our hypothesis that in ADPKD, multiple independent inactivating mutations in wild-type *PKD1* lead to polycystic kidneys. Somatic inactivation of both copies of *PKD1* may be the reason that approximately one of every four normal individuals has an isolated renal cyst by the age of 50 (24). The loss-of-function model may apply to *PKD2* (although we did not find chromosome 4q LOH in this study) and also to benign cystic tumors in other organs, including breast and ovary.

Increased levels of the *PKD1* protein, polycystin, which appear to be present in some renal cysts (25), would not be expected in the loss-of-function model since inactivating mutations usually decrease the half-life of both mRNA and protein products. However, inactivating *PKD1* mutations could prolong the half-life of polycystin, as has been demonstrated for the p53 tumor suppressor gene product (26, 27). It is also possible that PKD1 levels are normally regulated by a negative feedback mechanism and that the lack of this feedback in a cell with no functional polycystin leads to increased expression of mutant protein. This mechanism has been proposed to explain high levels of RNA expression of the Gorlin's syndrome gene in basal cell carcinomas with inactivating mutations in both alleles (28).

It is possible that somatic inactivation of the chromosome 16p13 tuberous sclerosis gene (*TSC2*), which is adjacent to *PKD1* (Fig. 2), contributes to cyst development in ADPKD. Renal cysts are a feature of tuberous sclerosis (29) and a contiguous gene syndrome of early-onset polycystic kidney disease has been described in infants with germline deletions of *PKD1* and *TSC2* (30). In the three cysts from patient 311 in which LOH is seen at both D16S283 and D16S525 (Table II), the chromosomal loss includes one copy of *TSC2* (Fig. 2). Loss of *TSC2* may also have occurred in the cysts from patients 168 and 305, in whom both D16S525 and D16S283 were not informative.

In conclusion, we developed two methods for isolating epithelial cells from renal cysts, and demonstrated loss of wild-type *PKD1* in these cells. The chromosomal alterations in cyst epithelial cells indicate that renal cysts are clonal lesions. Our data is consistent with a loss-of-function model for cyst development and suggests that renal cysts are benign epithelial neoplasms that fit Knudson's two-hit tumor suppressor gene model. Our finding of loss of chromosome 3p in one cyst could indicate a multi-step model of cyst pathogenesis, with inactivating mutations in other genes. We postulate that one function of polycystin is to suppress renal epithelial cell growth, and that inactivation of both copies of *PKD1* in a renal epithelial cell causes hyperplasia of that cell resulting in a cyst. Several studies have shown that epithelial hyperplasia is an important determinant of cyst enlargement (31–33). We found that LOH was not detectable when the entire cyst wall was used for the PCR assay, suggesting that only the epithelial cells, and not the other components of the cyst such as fibroblasts and inflammatory cells, are part of the neoplastic process. The loss-of-function model for cyst pathogenesis may suggest novel therapeutic approaches to delay or prevent end-stage renal failure in patients with ADPKD. These approaches could include the restoration of wild-type *PKD1* function in cyst epithelial cells to block further cyst growth, or the suppression of somatic mutations in the wild-type copy of *PKD1* to prevent cyst initiation.

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References

1. Gabow, P.A. 1993. Autosomal dominant polycystic kidney disease. *N. Engl. J. Med.* 329:332–342.

2. Peters, D.J.M., and L.A. Sandkuijl. 1992. Genetic heterogeneity of polycystic kidney disease in Europe. *Contrib. Nephrol.* 97:128–139.

3. Mochizuki, T., G. Wu, T. Hayashi, S.L. Xenophontos, B. Veldhuisen, J.J. Saris, D.M. Reynolds, Y. Cai, P.A. Gabow, A. Pierides, et al. 1996. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science (Wash. DC).* 272:1339–1342.

4. The European Polycystic Kidney Disease Consortium. 1994. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell.* 77:881–894.

5. The International Polycystic Kidney Disease Consortium. 1995. Polycystic kidney disease: the complete structure of the PKD1 gene and its protein. *Cell.* 81:289–298.

6. The American PKD1 Consortium. 1995. Analysis of the genomic sequence for the autosomal dominant polycystic kidney disease (PKD1) gene predicts the presence of a leucine-rich repeat. *Hum. Mol. Genet.* 4:575–582.

7. Hughes, J., C.J. Ward, B. Peral, R. Aspinwall, K. Clark, J.L. San Millan, and V. Gamble. 1995. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat. Genet.* 10:151–160.

8. Reeders, S.T. 1992. Multilocus polycystic kidney disease. *Nat. Genet.* 1: 235–237.

9. Grantham, J.J. 1990. Polycystic kidney disease: neoplasia in disguise. *Am. J. Kidney. Dis.* 15:110–116.

10. Weinberg, R.A. 1991. Tumor suppressor genes. *Science (Wash. DC).* 254:1138–1146.

11. Carone, F.A., S. Nakamura, B.S. Schumacher, P. Punyarit, and K.D. Bauer. 1989. Cyst-derived cells do not exhibit accelerated growth or features of transformed cells in vitro. *Kidney Int.* 35:1351–1357.

12. Shen, Y., H.M. Kozman, A. Thompson, H.A. Phillips, K. Holman, J. Nancarrow, S. Lane, L.-Z. Chen, S. Apostolou, N.A. Doggett, et al. 1994. A PCR-based genetic linkage map of human chromosome 16. *Genomics.* 22:68–76.

13. Snarey, A., S. Thomas, M.D. Schneider, S.E. Pound, N. Barton, A.F. Wright, S. Somlo, G.G. Germino, P.C. Harris, S.T. Reeders, and A.-M. Frischauf. 1994. Linkage disequilibrium in the region of the autosomal dominant polycystic kidney disease gene (PKD1). *Am. J. Hum. Genet.* 55:365–371.

14. San Millan, J.L., M. Viribay, B. Peral, I. Martinez, J. Weissenbach, and F. Moreno. 1995. Refining the localization of the PKD2 locus on chromosome 4q by linkage analysis in Spanish families with autosomal dominant polycystic kidney disease type 2. *Am. J. Hum. Genet.* 56:248–253.

15. Henske, E.P., B.W. Scheithauer, M.P. Short, R. Wollmann, J. Nahmias, N. Hornigold, M. vanSlegtenhorst, C.T. Welsh, and D.J. Kwiatkowski. 1996. Allelic loss is frequent in tuberous sclerosis kidney lesions but rare in brain lesions. *Am. J. Hum. Genet.* 59:400–406.

16. Henske, E.P., H.P.H. Neumann, B.W. Scheithauer, E.W. Herbst, M.P. Short, and D.J. Kwiatkowski. 1995. Loss of heterozygosity in the tuberous sclerosis (TSC2) region of chromosome band 16p13 occurs in sporadic as well as TSC-associated renal angiomyolipomas. *Genes Chromosomes and Cancer.* 13: 295–298.

17. Thrash-Bingham, C.A., H. Salazar, J.J. Freed, R.E. Greenberg, and K.D. Tartof. 1995. Genomic alterations and instabilities in renal cell carcinomas and their relationship to tumor pathology. *Cancer Res.* 55:6189–6195.

18. Fearon, E.R., and B. Vogelstein. 1990. A genetic model for colorectal tumorigenesis. *Cell.* 61:759–767.

19. Peral, B., J.L. San Millan, A.C.M. Ong, V. Gamble, C.J. Ward, C. Strong, and P.C. Harris. 1996. Screening the 3′ region of the polycystic kidney

disease 1 (PKD1) gene reveals six novel mutations. *Am. J. Hum. Genet.* 58:86–96.

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

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

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

23. Martin, G.M., C.E. Ogburn, L.M. Colgin, A.M. Gown, S.D. Edland, and R.J. Monnatt. 1996. Somatic mutations are frequent and increase with age in human kidney epithelial cells. *Hum. Mol. Genet.* 5:215–221.

24. Tada, S., J. Yamagishi, H. Kobayashi, Y. Hata, and T. Kobari. 1983. The incidence of simple renal cyst by computed tomography. *Clin Radiol.* 34:437–439.

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

26. Esrig, D., D. Elmajian, S. Groshen, J.A. Freeman, J.P. Stein, S.-C. Chen, P.W. Nichols, D.G. Skinner, P.A. Jones, and R.J. Cote. 1994. Accumulation of nuclear p53 and tumor progression in bladder cancer. *New. Engl. J. Med.* 331: 1259–1264.

27. Harris, C.C., and M. Hollstein. 1993. Clinical implications of the p53 tumor-suppressor gene. *New. Engl. J. Med.* 329:1318–1327.

28. Gailani, M.R., M.S. Stahle-Backdahl, D.J. Leffell, M. Glynn, P.G. Zaphiropoulos, C. Pressman, A.B. Unden, M. Dean, D.E. Brash, A.E. Bale, and R. Toftgard. 1996. The role of the human homologue of Drosophila patched in sporadic basal cell carcinomas. *Nat. Genet.* 14:78–81.

29. Bernstein, J., and T.O. Robbins. 1991. Renal involvement in tuberous sclerosis. *Ann. NY Acad. Sci.* 615:36–49.

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

31. Grantham, J.J., J.L. Geiser, and A.P. Evan. 1987. Cyst formation and growth in autosomal dominant polycystic kidney disease. *Kidney Int.* 31:1145–1152.

32. Bernstein, J., A.P. Evan, and K.D. Gardner. 1987. Epithelial hyperplasia in human polycystic kidney disease. *Am. J. Path.* 129:92–101.

33. Wilson, P.D. 1991. Aberrant epithelial cell growth in autosomal dominant polycystic kidney disease. *Am. J. Kidney Dis.* 17:634–637.