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Renal Cystic Disease in Tuberous Sclerosis: Role of the Polycystic Kidney Disease 1 Gene

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Summary

Tuberous sclerosis is an autosomal dominant trait characterized by the development of hamartomatous growths in many organs. Renal cysts are also a frequent manifestation. Major genes for tuberous sclerosis and autosomal dominant polycystic kidney disease, *TSC2* and *PKD1*, respectively, lie adjacent to each other at chromosome 16p13.3, suggesting a role for *PKD1* in the etiology of renal cystic disease in tuberous sclerosis. We studied 27 unrelated patients with tuberous sclerosis and renal cystic disease. Clinical histories and radiographic features were reviewed, and renal function was assessed. We sought mutations at the *TSC2* and *PKD1* loci, using pulsed field– and conventional-gel electrophoresis and FISH. Twenty-two patients had contiguous deletions of *TSC2* and *PKD1*. In 17 patients with constitutional deletions, cystic disease was severe, with early renal insufficiency. One patient with deletion of *TSC2* and of only the 3′ UTR of *PKD1* had few cysts. Four patients were somatic mosaics; the severity of their cystic disease varied considerably. Mosaicism and mild cystic disease also were demonstrated in parents of 3 of the constitutionally deleted patients. Five patients without contiguous deletions had relatively mild cystic disease, 3 of whom had gross rearrangements of *TSC2* and 2 in whom no mutation was identified. Significant renal cystic disease in tuberous sclerosis usually reflects mutational involvement of the *PKD1* gene, and mosaicism for large deletions of *TSC2* and *PKD1* is a frequent phenomenon.

Introduction

Tuberous sclerosis is an autosomal dominant trait recognized particularly for its neurological and dermatological manifestations. These include seizures and learning difficulties, facial angiofibromas (adenoma sebaceum), periungual fibromas, shagreen patches, and hypopigmented macules (Gomez 1988). Renal manifestations are common and include angiomyolipomas, cystic disease, and, exceptionally, renal cell carcinoma (Cook et al. 1996). In patients with tuberous sclerosis, renal causes of death are second only to CNS causes (Shepherd et al. 1991). The radiological and macroscopic appearances of cystic disease resemble autosomal dominant polycystic kidney disease (ADPKD) (Torres et al. 1994). However, clinical onset is often early (Wenzl et al. 1970; Stapleton et al. 1980; Campos et al. 1993; Webb et al. 1993; Cook et al. 1996), and the hypertrophic eosinophilic cells of the cystic epithelium are considered to be unique, by some authors (Elkin and Bernstein 1969; Bernstein 1993).

Two tuberous sclerosis genes, termed *"TSC1"* and *"TSC2,"* have been localized to chromosomes 9q34 (Fryer et al. 1987) and 16p13.3 (Kandt et al. 1992), respectively. The *TSC2* gene has been identified and characterized (European Chromosome 16 Tuberous Sclerosis Consortium 1993). It lies immediately adjacent to *PKD1*, the major gene for ADPKD (European Polycystic Kidney Disease Consortium 1994), raising the possibility that *PKD1* plays a role in the etiology of renal cystic disease in tuberous sclerosis. We previously investigated 6 patients with tuberous sclerosis and severe polycystic kidney disease who had presented during the first few months of life (Brook-Carter et al. 1994). In each case, a submicroscopic deletion at chromosome 16p13.3, involving both the *TSC2* and the *PKD1* genes, was identified. In order to establish whether *PKD1* plays a more general role in renal cystic disease in tuberous sclerosis, we have now investigated 27 unrelated patients with tuberous sclerosis and renal cystic disease of varying degrees of severity.

Patients and Methods

Patients

We studied 27 unrelated patients with tuberous sclerosis and multiple bilateral renal cysts (table 1). Eighteen patients were identified via the Tuberous Sclerosis Association or via nephrology or clinical genetics units,

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Table 1

Presentation of Renal Cystic Disease

Subject	Sex	Age at Diagnosis	Presentation or Method of Diagnosis
TSC2/PKD1-deletion case:			
1	Male	3 mo	Abdominal masses
2	Male	1 mo	Abdominal distention
3	Male	4 mo	Abdominal masses
4	Male	6 mo	Abdominal distention
5	Male	5 mo	Abdominal masses
6	Male	6 mo	Abdominal masses
7	Male	1 mo	Abdominal masses
8	Male	2 years	Abdominal masses
9	Female	6 mo	Abdominal masses
10	Male	7 mo	Abdominal distention
11	Male	8 mo	Abdominal masses
12	Male	10 years	Ultrasound screening
13	Female	21 mo	Ultrasound screening
14	Male	3 mo	Abdominal masses
15	Male	6 mo	Abdominal distention
16	Female	4 mo	Abdominal distention
17	Female	7 mo	Ultrasound screening
PKD1 3′-UTR deletion case:			
18	Female	45 years	Ultrasound screening
Mosaic case:			
19	Female	5 years	Hematuria, abdominal masses
20	Male	5 mo	Abdominal distention
21	Female	5 years	Abdominal masses
22	Female	3 years	Abdominal masses
Other cases (mutation):			
23 (*TSC2* deletion)	Female	37 years	Ultrasound screening
24 (*TSC2* deletion)	Female	35 years	Ultrasound screening
25 (inversion)	Female	2 years	Ultrasound screening
26 (no mutation identified)	Male	13 years	Ultrasound screening
27 (no mutation identified)	Female	27 years	Ultrasound screening
Mosaic parent of *TSC2/PKD1*-deletion cases:			
Mother of patient 7	...	37 years	Ultrasound screening
Father of patient 12	...	35 years	Ultrasound screening
Father of patient 13	...	30 years	Ultrasound screening

through a call for patients with tuberous sclerosis and renal cystic disease. Six of these cases have been reported elsewhere (patients 1 [WS-53], 2 [WS-194], 3 [WS-215], 4 [WS-219], 5 [WS-227], and 6 [WS-250]; Brook-Carter et al. 1994). Nine additional patients were identified from among a cohort of 90 sequentially ascertained patients with tuberous sclerosis who underwent prospective ultrasound assessment for renal involvement, in the absence of signs or symptoms of renal disease. Twenty-four of the 27 cases were apparently sporadic, with no family history of either tuberous sclerosis or polycystic kidney disease. Three cases had parents with tuberous sclerosis and cystic kidneys.

Clinical Evaluation

All patients fulfilled the definitive diagnostic criteria for tuberous sclerosis, described by Gomez (1988) and by the Diagnostic Criteria Committee of the National Tuberous Sclerosis Association (Roach et al. 1992). Renal ultrasound films were reviewed, and the presence or absence, the laterality, and the multiplicity of renal cysts and of angiomyolipomas were recorded. In cases for whom assessment of renal cystic disease was problematic, owing to, for example, coexisting renal angiomyolipomas, abdominal computed tomograms and magnetic nuclear-resonance images were obtained and were reviewed. Plasma creatinine levels were measured, and estimates of the glomerular filtration rate (GFR) based on the clearance of creatinine, of Tc^{99}DPTA (diethylenetriaminepentaacetic acid) or of Cr^{51}EDTA, were recorded. When this was not possible, the GFR was estimated from plasma creatinine levels by use of the Schwartz formula (Schwartz et al. 1976), for those patients <17 years of age, or the formula of Cockcroft and Gault (1976), for adults.

Identification and Characterization of Mutations

We have constructed previously a restriction map around the *TSC2* and *PKD1* loci and have described most of the probes used in this study (European Chromosome 16 Tuberous Sclerosis Consortium 1993; European Polycystic Kidney Disease Consortium 1994; Hughes et al. 1995). The newly described probe BFS5 is a 4.3-kb *NotI*/*Hind*III fragment of probe CW23. For PFGE analysis of patient samples, high-molecular-weight DNA was prepared from peripheral blood leukocytes, in agarose plugs (Hermann et al. 1987), was digested with the appropriate restriction enzymes, and was resolved by use of a BioRad CHEF DRII apparatus and 1% agarose gels. For conventional gel electrophoresis, DNA was extracted by standard methods (Sambrook et al. 1989), was digested with various enzymes, and was resolved by use of 0.5%–1.0% agarose gels. Blotting and hybridization were by conventional methods (Sambrook et al. 1989). A phosphoimager (STORM 860; Molecular Dynamics) was used to quantify the breakpoint-fragment intensity in patient F13.

A general strategy for detection of deletions at the *TSC2* and *PKD1* loci, by use of pulsed field–gel electrophoresis (PFGE), has been described elsewhere (Brook-Carter et al. 1994). Restriction fragments that span the area were assayed with probes located proximal to and distal to the genes. Abnormal fragments detected by flanking probes, but not by one or more probes within the genes, were considered to be indicative of deletions. The precise areas deleted then were established by hybridization of further genomic and cDNA fragments from between the flanking probes. Deletions >10–20 kb could be detected by this method. Smaller deletions were sought by conventional gel electrophoresis, with concentration on the adjacent 3′ regions of the *TSC2* and *PKD1* genes.

FISH was undertaken by use of probes mapping within the deleted areas. This enabled confirmation of deletion mutations and quantification of somatic mosaicism. The methods used have been described elsewhere (Brook-Carter et al. 1994).

Sequencing of the Breakpoint in Patient 16

Primers for intron 40 of *TSC2* (1316D; 5′-CAAGCCGCCTCTGCCTTC-3′) and exon 11 of *PKD1* (1316P; 5′-TGACGTGGTCTCCCCAGTGG-3′) were designed to amplify a breakpoint fragment from genomic DNA of patient 16. PCR buffers and conditions were as described elsewhere (Harris et al. 1991). The resulting fragment of 238 bp was cloned in pZErO and was sequenced.

Results

Genetic Analysis

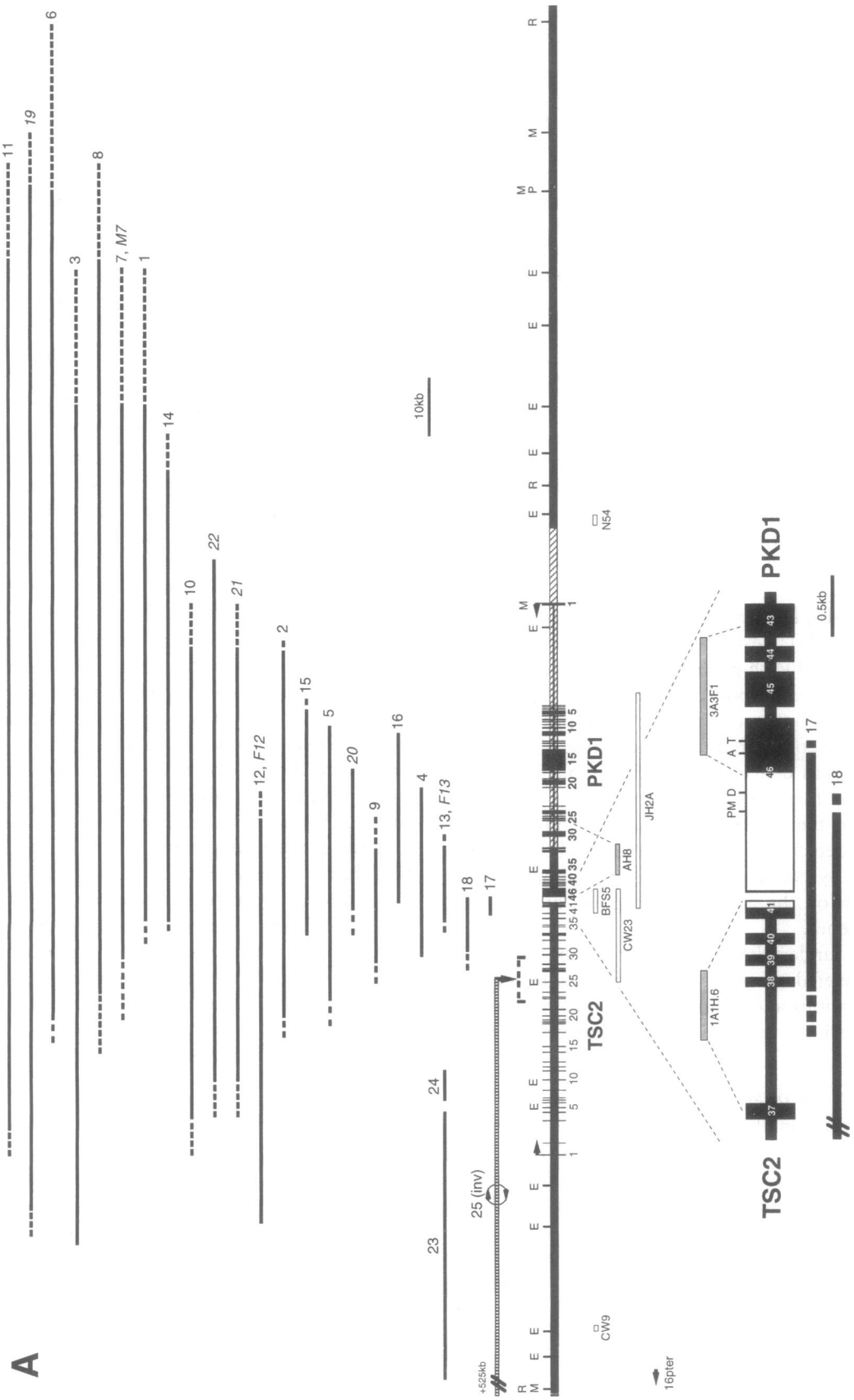
Deletions of TSC2 and PKD1.—DNA deletions disrupting both *TSC2* and *PKD1* were identified in 22/27 patients (fig. 1). Most deletions removed substantial portions or all of both genes. However, in one case (patient 16), the probe (BFS5) to the 3′ ends of the *PKD1* and *TSC2* genes was partially present. To determine if the *TSC2* gene was disrupted, a breakpoint fragment was cloned and was sequenced (see Patients and Methods for details); 84 bp of the *TSC2* coding sequence were deleted. In patients 17 and 18, the deletions involved only the most 3′ part of *PKD1*. The breakpoint in patient 17 was localized within the coding region of the final exon of *PKD1*, whereas the breakpoint in patient 18 was localized within the 3′ UTR.

Deletions and an inversion disrupting TSC2.—Gross rearrangements of *TSC2* without *PKD1* involvement were found in three patients (fig. 1*A*). Two of these deletions in *TSC2* have been reported elsewhere (for patients 23 [WS-9] and 24 [WS-11]; European Chromosome 16 Tuberous Sclerosis Consortium 1993). The third case, patient 25, was shown to have an inversion of ≈600 kb of DNA, by use of a combination of PFGE and FISH analysis. The proximal breakpoint was within *TSC2*, and, therefore, the inverted DNA segment lay entirely distal to *PKD1* (fig. 1*A*).

Somatic mosaicism.—In patients 19, 20, 21, and 22, autoradiography revealed breakpoint fragments of lower signal intensity than that of their normal counterparts. This suggested that the patients might be somatic mosaics, with only a proportion of their cells carrying a deleted chromosome. FISH analysis using probes mapping within the deletions confirmed and quantified mosaicism (fig. 2 and table 2). PFGE and FISH analysis of samples from the father of patient 12 (individual F12) and the mother of patient 7 (individual M7) showed that they also were mosaics and that their deleted chromosomes had been transmitted to their offspring (fig.1 and table 2). PFGE analysis of the father of patient 13 (individual F13) also suggested mosaicism, but, since no probe suitable for FISH was deleted completely, the frequency of the mutant allele was assessed by measurement of the signal from a *Bam*HI breakpoint fragment, on a Southern blot detected by probe CW23. The mutant allele was estimated to be present in 15% of leukocytes.

Clinical Evaluation

Cases with deletions involving TSC2 and PKD1.—The presentation and course of renal cystic disease in the 17 patients with nonmosaic deletions of the coding regions of *TSC2* and *PKD1* were very similar (table 1), although the nonrenal manifestations of tuberous sclerosis varied considerably. All 17 patients were found to have enlarged cystic kidneys during infancy or childhood, with radiographic features resembling advanced ADPKD, at the time of diagnosis (fig. 3). Five patients presented with cystic kidneys before other features of tuberous sclerosis developed, leading to incorrect initial diagnoses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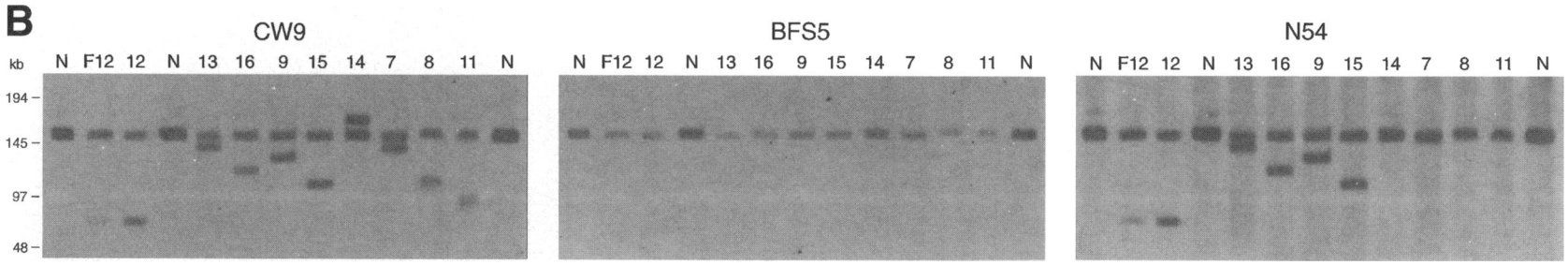

Figure 1 Deletions and inversion in patients with tuberous sclerosis and renal cystic disease. *A*, Map of the *TSC2/PKD1* region of chromosome 16. The genomic map is shown as a blackened bar, with restriction sites for *Eco*RI ("E"), *Mlu*I ("M"), and *Nru*I ("R") indicated. The hatched region indicates the part of the *PKD1* locus that is duplicated elsewhere on chromosome 16. The locations of exons of the *PKD1* gene and the *TSC2* gene are shown, and the directions of transcription are indicated by arrows. The genomic probes (unblackened boxes) and the cDNA probes (hatched boxes) that were used for PFGE (panel *B*) and for FISH are shown below the map. The regions of DNA deleted in tuberous sclerosis patients with renal cystic disease are shown above the map. The solid lines indicate the regions definitely deleted, and the dashed lines indicate regions of uncertainty. The patients' numbers are indicated, and the patients who are mosaic for deletions are shown in italics. The proximal inversion breakpoint in patient 25 is illustrated, and the distal breakpoint lies ≈600 kb telomeric to TSC2. The 3′ ends of the *PKD1* and *TSC2* genes are expanded *below* the diagram. The cDNA probes and the restriction sites used to localize the proximal breakpoints in patients 17 and 18 are indicated. The proximal deletion breakpoint in patient 17 was localized between *Alw*I ("A") and *Taq*I ("T") sites within the coding region of *PKD1*, whereas the proximal breakpoint in patient 18 mapped within the 3′ UTR, between *Dra*I ("D") and *Pfl*MI ("PM") sites. *B*, Examples of PFGE, to detect deletions in patients with tuberous sclerosis and renal cystic disease. *Nru*I-digested DNA from a normal control (lane N) and from patients (numbered lanes) were hybridized with a probe (CW9) distal to *TSC2* (*left*); with a probe (BFS5) containing the 3′ regions of *TSC2* and *PKD1* (*middle*), and with a probe (N54) proximal to *PKD1* (*right*) (see panel *A* for probe locations). Each of the probes detected the same normal fragment of 155 kb. An additional fragment is seen in all patients, by use of CW9, whereas BFS5 is deleted completely in all cases, except in patient 16, for whom the breakpoint is in the last exon of *TSC2*. N54 lies proximal to the deletions in patients 12, 13, 16, 9, and 15 but is deleted in patients 14, 7, 8, and 11; the proximal extent of the deletions in these cases was defined with other probes, from the adjacent *Nru*I fragment (data not shown). An example of somatic mosaicism is illustrated on the *left* side of the gel. The breakpoint fragment seen in patient 12 also is seen, but at reduced intensity, in the patient's father (lane F12), suggesting that the deleted chromosome was present in only a proportion of white blood cells. Mosaicism was confirmed and quantified by FISH (table 2).

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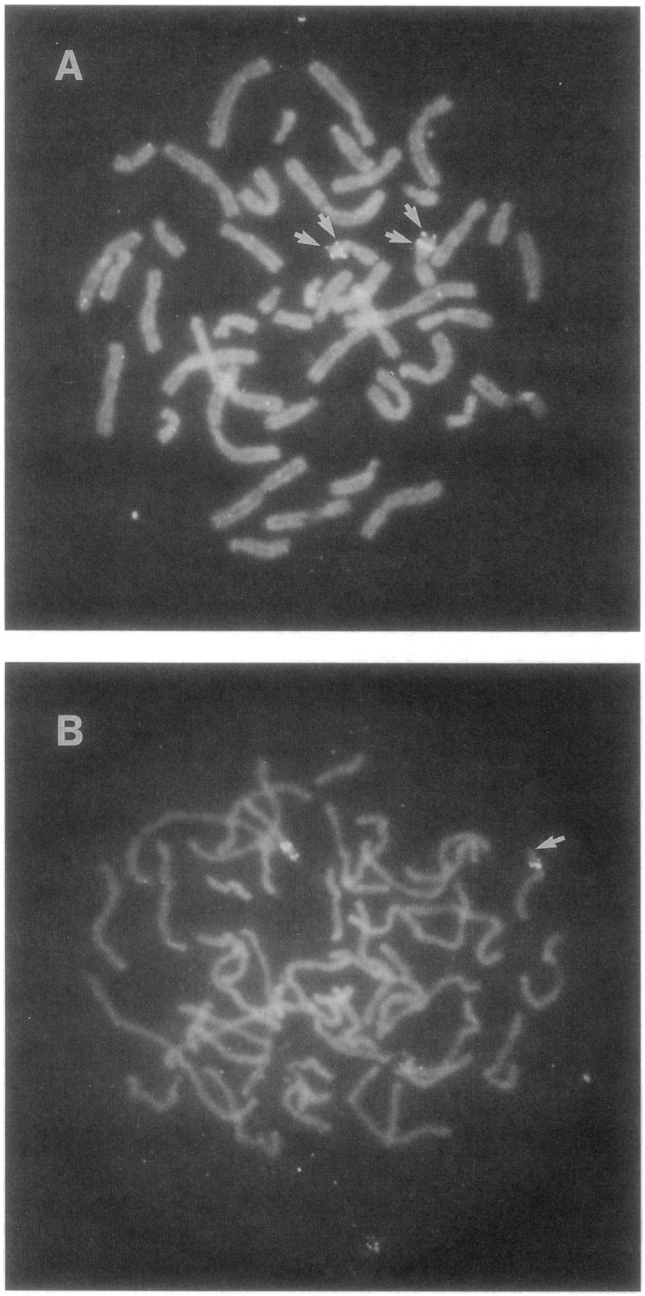

Figure 2 FISH analysis for patient 19. Cosmid JH2A hybridized to different metaphase spreads. The cosmid hybridized to the distal *TSC2/PKD1* locus and, more strongly, to the homologous multiple-copy proximal locus. *A*, Nondeleted cell, with proximal and distal signals indicated by arrows. *B*, Cell deleted at the *TSC2/PKD1* locus (indicated by an arrow). In patient 19, 35% of cultured lymphocytes were deleted for JH2A (table 2).

of early onset ADPKD or of autosomal recessive polycystic kidney disease. Serial radiography revealed increasing cyst size and reduction of residual parenchyma, in some patients. Small echogenic areas consistent with angiomyolipomata developed in 2 patients.

Twelve patients required antihypertensive treatment. GFRs were normal or only slightly reduced among patients still in the first decade of life. GFRs were markedly reduced in most older patients, and the 3 oldest patients had reached end-stage renal disease prior to enrollment, at the ages of 19, 20, and 29 years (fig. 4). One patient was treated successfully by hemodialysis, another by chronic ambulatory peritoneal dialysis, and the third did

Table 2

FISH in Patients with Somatic Mosaicism for *TSC2/PKD1* Deletions and in Normal Controls

Subject(s)	Probe[a]	Percentage of Cells with Signal on Chromosome 16, on[b]: Both Homologues	One Homologue
Patient:			
19	JH2A	65	35
20	AH8	76	34
21	JH2A	66	34
22	JH2A	39	61
M7	JH2A	58	42
F12	CW23	76	24
Controls:			
1/2/3	JH2A	95/96/97	5/4/3
1/2/3	AH8	89/88/95	11/12/5
1/2/3	CW23	95/92/94	5/8/6

[a] Positions of probes used for FISH are shown in figure 1 relative to the positions of deletions.

[b] One hundred cells were scored per patient, except for patient 19, in whom 26 cells were scored.

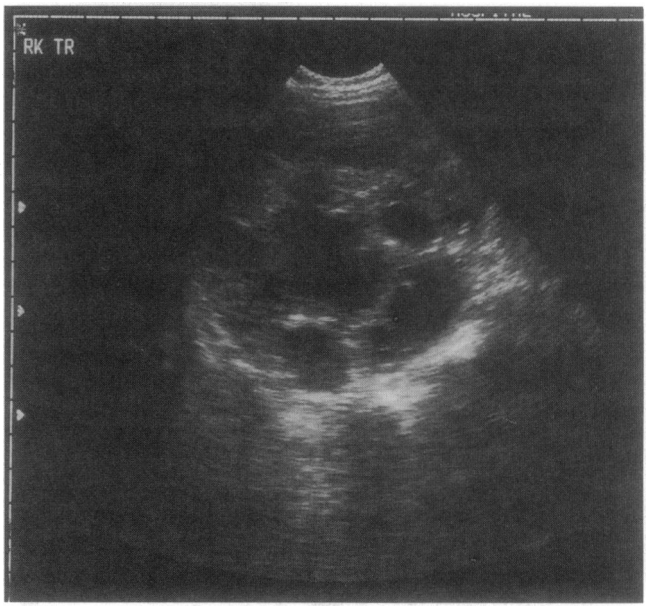

Figure 3 Result of renal ultrasound for patient 7 (6 years of age). The transverse section through the right kidney shows multiple cystic areas throughout and largely replacing the kidney.

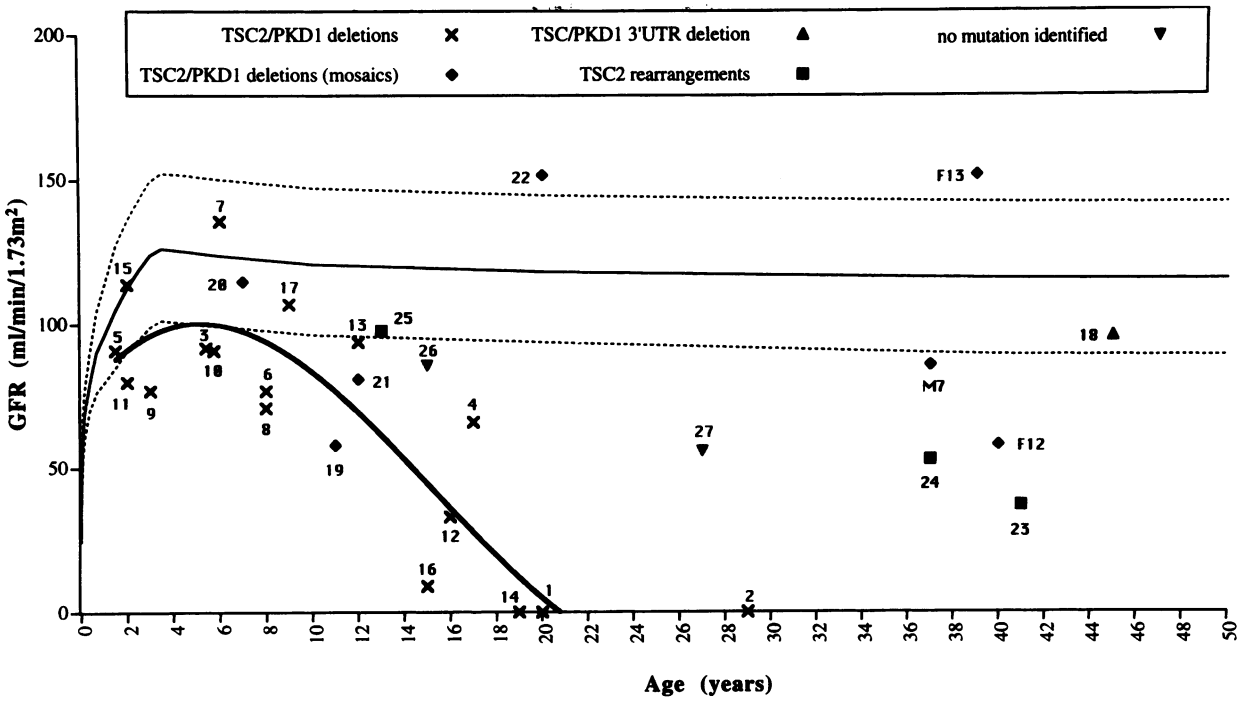

Figure 4 GFRs in 30 patients with tuberous sclerosis and renal cystic disease. For each patient, the most recent estimate of GFR, corrected for body surface area, is plotted relative to a nomogram of GFRs corrected for body surface area at different ages (mean and ±2 SD; from McCrory 1972). Patients are identified by their individual numbers (1–27), and GFRs in the affected parents of patients 7, 12, and 13 are indicated as "M7," "F12," and "F13," respectively. The curve of best fit is for cases with constitutional deletions involving the coding regions of *TSC2* and *PKD1*.

not receive medical intervention and died. Bilateral open renal biopsy was undertaken for patient 16. Pathological examination revealed many cysts, ranging in size from microscopic to >1 cm in diameter. The histopathological features (fig. 5) were indistinguishable from those described elsewhere for renal cystic disease in tuberous sclerosis (Bernstein 1993).

Mosaics.—The severity of renal cystic disease and of other features of tuberous sclerosis varied widely among the seven patients with mosaicism for deletions involving *TSC2* and *PKD1*. Cystic disease in the three mosaic parents was recognized only in adult life, following diagnosis of their more severely affected children. The radiographic appearances were similar to those of the constitutionally deleted cases, but renal function was better preserved (fig. 4).

Deletion of the 3′ UTR of PKD1.—In patient 18, the deletion involved *TSC2* but only the 3′ UTR of *PKD1*. The patient had adenoma sebaceum and periungual fibromas, which are skin stigmata that are both diagnostic of tuberous sclerosis. At 45 years of age, the patient underwent prospective renal ultrasound and was found to have a few cysts and multiple angiomyolipomas, in both kidneys. Renal function was normal.

Cases without PKD1 involvement.—In five patients there was no evidence of structural disruption of *PKD1*. Patients 23 and 24, with large deletions involving *TSC2* but not *PKD1*, had numerous angiomyolipomata and a small number of cysts, in each kidney. Mixed angiomyolipomata and cysts also were present in patients 26 and 27, in whom no mutation could be identified. Moder-

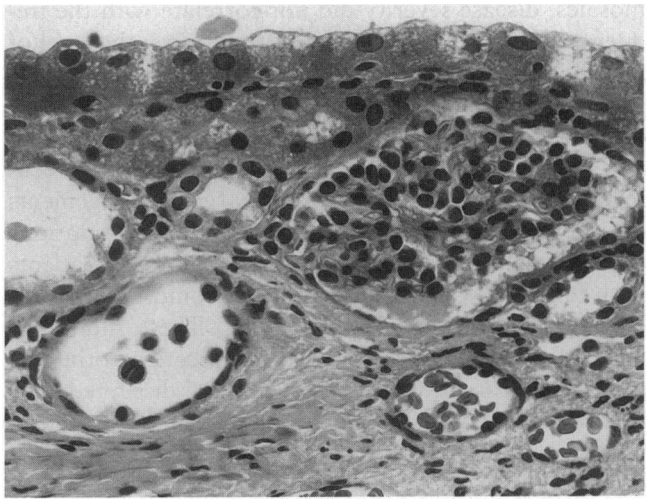

Figure 5 Renal histopathology in patient 16. The hematoxylin- and eosin-stained section is shown at 500 × magnification. The cystic epithelium (*top*) comprises large cells with strongly eosinophilic and abundant cytoplasm. Mitotic figures and piling up of the epithelium into small mounds also were seen occasionally (not shown).

ately severe renal cystic disease was present in patient 25, who had an inversion disrupting *TSC2* but not *PKD1*.

Discussion

A possible role for *PKD1* in renal cystogenesis in tuberous sclerosis was suggested by Kandt et al. (1992) when *TSC2* was mapped to the region of chromosome 16 containing *PKD1*. Once isolated, *TSC2* and *PKD1* were shown to lie immediately adjacent to one another, and deletions involving both genes were identified in six patients with tuberous sclerosis and severe early onset polycystic kidney disease (Brook-Carter et al. 1994). The present study establishes a more general role for *PKD1* in the etiology of renal cystic disease in tuberous sclerosis. This may be mediated via a number of mutational mechanisms, apparently accounting for at least some of the observed variation in disease severity. In this series, constitutional deletions involving the coding regions of both *TSC2* and *PKD1* were associated consistently with severe early onset renal cystic disease, with renal enlargement and radiological appearances similar to those with advanced ADPKD. We did not identify any similar deletions among 81 unrelated patients with tuberous sclerosis but without evidence of renal cystic disease, as determined by an ultrasound scan (authors' unpublished data). The cross-sectional data presented in this study suggest that the prognosis for renal function is poor in cases with constitutional deletions involving *TSC2* and *PKD1*. However, most of the patients studied were young, and long-term follow-up is required in order to define the natural history of the renal disease in this group. Mosaicism for deletions involving *TSC2* and *PKD1* was a frequent phenomenon and was associated with preserved renal function in some cases. Among mosaics, disease severity did not correlate with the frequency of the mutant allele in lymphocytes; the level of mosaicism in renal tissue is likely to be more important. Five of the 27 unrelated patients studied had multiple cysts in both kidneys, but no detectable disruption of *PKD1*. All were identified, through ultrasound screening, as having renal cystic disease. Large rearrangements of *TSC2* were defined in 3 of these patients. Mutations of this type have been demonstrated in very few patients with tuberous sclerosis (European Chromosome 16 Tuberous Sclerosis Consortium 1993). These mutations may affect *PKD1* expression, if enhancers or other remote regulatory sequences of *PKD1* are disrupted. The failure to identify any mutation in 2 of the 27 cases might reflect the sensitivity of the assays used, since low-level or tissue-specific mosaicism could not be excluded. It remains possible that mild cystic disease could be associated with subtle mutations of *TSC2* or of *TSC1*, without *PKD1* involvement. However, significant cystic disease has not been documented in affected members of *TSC1* (chromosome 9 linked) families.

We found that the histopathological features of renal cystic disease in patient 16, who had a deletion involving *TSC2* and *PKD1*, were indistinguishable from those in previously reported cases (Elkin and Bernstein 1969; Bernstein 1993) in whom the mutational basis had not been determined. It is likely that these cases also had deletions involving both genes. Renal cystic disease associated with the contiguous deletion of *TSC2* and *PKD1* appears to differ from ADPKD, both histopathologically and in terms of age of onset and severity. This could reflect the combined effects of mutation of *TSC2* and *PKD1* in the developing kidney. The nature of the *PKD1* mutation also could be important. The large deletions that we identified in patients with tuberous sclerosis can be expected to inactivate *PKD1*. In contrast, the consequences of the more subtle mutations so far identified in patients with ADPKD (Peral et al. 1995, 1996; Turco et al. 1995) are less clear, and, perhaps surprisingly, both overexpression of the *PKD1* product, polycystin, and loss of heterozygosity have been reported in the cystic epithelium of ADPKD patients (Qian et al. 1996; Ward et al. 1996; Brasier and Henske 1997).

In contrast to the unpredictable nature of many complications of tuberous sclerosis, it can be reasoned that renal cystic disease due to deletion of *PKD1* will "breed true." Familial occurrence of renal cystic disease in tuberous sclerosis already has been noted (Cree 1969; O'Callaghan et al. 1975), and molecular investigation of familial cases now is warranted. Testing for deletions at the *TSC2* and *PKD1* loci also should prove useful during investigation of polycystic kidney disease detected in infancy (or even antenatally). In the past, the initial absence of other features of tuberous sclerosis frequently had led to diagnostic confusion (Wenzl et al. 1970; Stapleton et al. 1980; Webb et al. 1993; Brook-Carter et al. 1994). This now can be avoided, by means of clinical awareness and appropriate molecular investigation. Characterization of the deletions also may be of prognostic value. The data presented here suggest that progression to end-stage renal disease in late childhood or in early adult life may be anticipated for cases with inactivation of *PKD1*, whereas a more hopeful prognosis may be appropriate when 3′-UTR involvement or mosaicism can be demonstrated.

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