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Genetic renal disease

Original article

Diffuse renal cystic disease in children: morphologic and genetic correlations

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Abstract. During a 5-year period, we evaluated seven infants and two fetuses who presented with enlarged, hyperechoic kidneys. In each, the initial clinical diagnosis was autosomal recessive polycystic kidney disease (ARPKD). Among the seven unrelated infants were three Caucasian and four African-American infants. No syndromic stigmata were evident in any of these infants. At the time of the initial evaluation, the family data were incomplete for four infants. The two fetuses were presumed to be at-risk for ARPKD based on the diagnosis in previous siblings. Renal histopathology was evaluated in all nine cases and revealed a spectrum of cystic disease ranging from ARPKD to glomerulocystic kidney disease to autosomal dominant polycystic kidney disease to diffuse cystic dysplasia. In the eight cases for whom liver histopathology was available, varying degrees of biliary dysgenesis were evident. We present a detailed analysis of the key histopathological features in each case and discuss the histopathological findings in an embryological context. In addition, we address the current role of molecular genetics in the diagnostic evaluation.

Key words: Renal cystic disease – Autosomal recessive polycystic kidney disease – Autosomal dominant polycystic kidney disease – Glomerulocystic kidney disease – Meckel syndrome – Molecular analysis

Introduction

Diffuse renal cystic diseases comprise a complex group of disorders which often present in the perinatal period or the first few years of life. Although these disorders are genetically distinct, they have overlapping clinical, radiographic, and morphological features, and are variably associated with biliary dysgenesis. The sonographic hallmark of renal cystic disease is enlarged, diffusely echogenic kidneys with loss of corticomedullary differentiation. Numerous clinical reports have established that this sonographic pattern is commonly seen in autosomal recessive polycystic kidney disease (ARPKD), autosomal dominant polycystic kidney disease (ADPKD), glomerulocystic kidney disease (GCKD), and diffuse cystic dysplasia [1, 2]. Sonographic evaluation, family history, and histopathological analysis have been mainstays in the diagnostic evaluation of diffuse renal cystic disease. Recent genetic advances have begun to add molecular tools, such as genetic linkage analysis, to this diagnostic armamentarium.

We have evaluated nine infants who presented either in utero or within the first 2 years of life with enlarged, hyperechoic kidneys and various degrees of biliary dysgenesis. Detailed family histories were taken at the time of the initial evaluation and subsequently at 1- to 2-year intervals. From a histological perspective, these cases include the common forms of diffuse renal cystic diseases. We present a detailed analysis of the key histopathological features in each case and discuss the histopathological findings in an embryologic context. Where possible, we have applied molecular genetic tools to help establish the diagnosis.

Patients and methods

Patients. Seven infants presented for evaluation at either the Children's Hospital of Alabama or the University of Vermont. As summarized in Table 1, all of the children presented at less than 2 years of age with enlarged, echogenic kidneys. In addition, the two fetal cases (patients 8 and 9) were evaluated. Patient 8 was at risk for ARPKD given autopsy-proven disease in an older sibling. The family had requested haplotype-based prenatal analysis for ARPKD in this second pregnancy. In the family of patient 9, there was some diagnostic uncertainty. A previous sibling had presented with the Potter sequence at birth and diffuse renal cystic disease. No other abnormalities were noted on physical examination and an autopsy was not performed. In patient 9, our review of the autopsy report revealed a renal cystic lesion as well as an occipital encephalocele. The renal histopathology in this fetus

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Table 1. Initial clinical data

Patient no.	Gender	Race	Age at presentation	Initial parental renal sonogram	Age at biopsy/autopsy	Liver histopathology
1	F	AA	32 weeks' GA	Negative; parents in mid-twenties	1 day	+
2	F	C	Birth	Negative; parent >30 years old	1 month	+
3	F	C	31 weeks' GA	Negative; parents >30 years old	2 years	+
4	F	AA	5 months	Negative; parents >30 years old	6 months	Not done
5	F	AA	17 months	Negative; mother in mid-twenties	17 months	+
6	M	C	2 weeks	Negative; parents ~20 years old	3 months	+
7	M	AA	34 weeks' GA	Negative; parents >30 years old	34 weeks' GA	+
8	M	C	21 weeks' GA	Not done	21 weeks' GA	+
9	M	C	18 weeks' GA	Not done	18 weeks' GA	+

C, Caucasian; AA, African-American; GA, gestational age

was reviewed to determine whether haplotype-based prenatal analysis for ARPKD was appropriate for the family's planned future pregnancy. In all patients, the kidney was evaluated by light microscopy. Liver histology was evaluated in all but patient 4.

Genetic evaluation. To assess linkage to the principal ADPKD locus, *PKD1*, four polymorphic microsatellites: *D16S85, KG8CA, D16S665 (SM6),* and *D16S291*, were typed in family 2. Of these microsatellite markers, *D16S85* lies telomeric to the gene, *KG8CA* is contained within the 3' untranslated region of the *PKD1* gene, *D16S665* lies just proximal to the 5' end, and *D16S291* lies approximately 100 kilobases centromeric [3, 4]. In addition, three microsatellite markers linked to *PKD2* were typed in this family. *D4S395* and *D4S423* are respectively centromeric and telomeric flanking markers and *D4S1534* is closely linked to the locus [5–7].

In family 8, known to be at-risk for ARPKD, haplotype-based prenatal analysis was performed. The markers, *D6S269 - D6S272 - D6S465 - D6S466 - D6S295*, span the candidate region, and the ARPKD locus lies between *D6S465* and *D6S466* [8].

DNA was obtained from these families with informed consent. For living pedigree members, DNA was extracted from peripheral blood lymphocytes according to standard protocols. For the deceased affected child, DNA was extracted from paraffin-embedded tissue as previously described [8]. All microsatellite markers were examined by polymerase chain reaction amplification [8, 9]. The amplified fragments were separated on 6% polyacrylamide sequencing gels and the results analyzed by autoradiography. Haplotypes were constructed from the genotype data.

Results

Initial clinical data

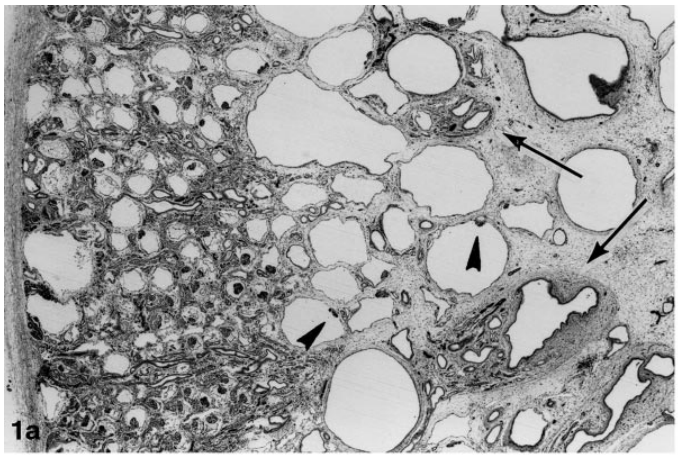
As summarized in Table 1, seven children presented for evaluation of enlarged kidneys at less than 2 years of age. Most were diagnosed either in utero or in the perinatal period. No evidence of syndromic stigmata was evident on physical examination. Renal sonography revealed varying degrees of nephromegaly and increased echogenicity.

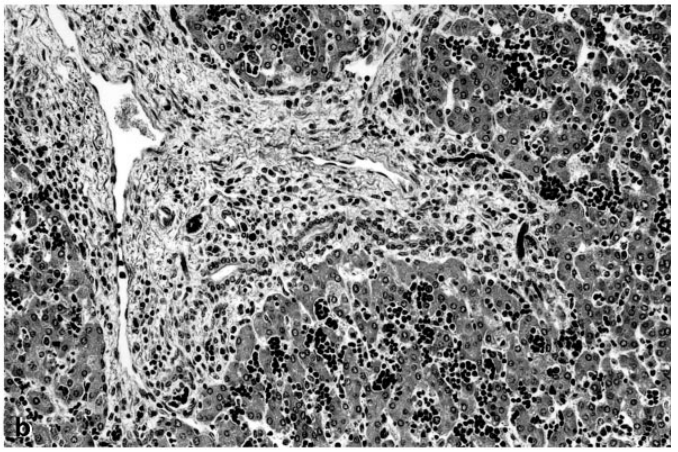
Patients 1 and 7 died within 24 h of birth secondary to respiratory insufficiency. Patient 2 presented with enlarged, echogenic kidneys at 1 month of age. There was no evidence of renal cystic disease in her then 34-year-old mother or 42-year-old father. Patient 3 was noted to have symmetrically enlarged, echogenic kidneys at 31 weeks' gestational age. Her fraternal twin had a "grossly cystic" right kidney and an enlarged, echogenic left kidney with poor corticomedullary differentiation. Shortly after the twins' birth, their parents underwent renal sonographic evaluation. These studies were reported to be normal. The renal sonograms were also normal in the parents of patient 4 and 6. Initially, only patient 5's mother was available for sonographic evaluation and these studies were normal. Patients 8 and 9 had previous siblings who died in the perinatal

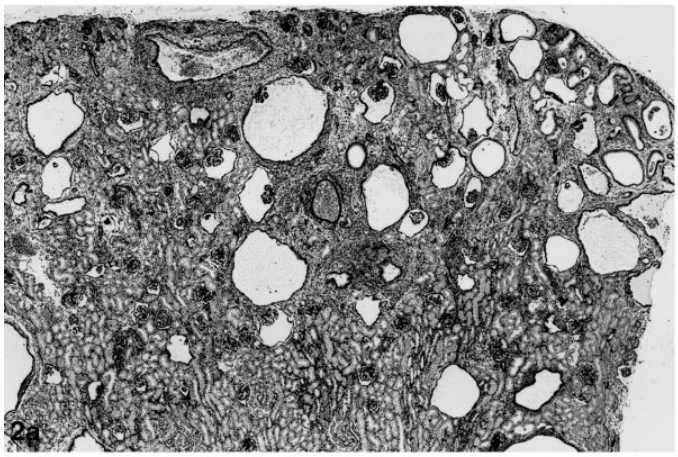
Table 2. Clinical outcome

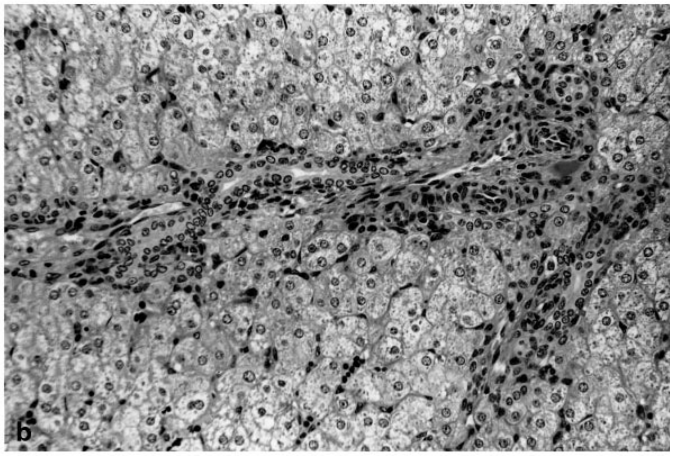
Patient no.	Clinical follow-up	Family ultrasound (US) follow-up	Pathological diagnosis	Genetic evaluation	Final diagnosis
1	Death at <24 h	–	GCKD	–	GCKD
2	Normal GFR at 2 years; hypertension	Paternal grandmother's renal US: ADPKD	GCKD	Consistent with *PKD2*	GCKD
3	Normal GFR at 4 years; normal blood pressure	US re-review: mother has 1 renal cyst	GCKD	–	GCKD
4	Normal GFR at 6 years; hypertension	–	ADPKD	–	ADPKD
5	30% GFR at 6 years; hepatosplenomegaly	Father's renal US: ADPKD	ARPKD	Paternity confirmed	?ADPKD
6	Normal GFR at 5 years; hypertension	–	ARPKD	–	ARPKD
7	Death at <12 h	–	ARPKD	Consistent with ARPKD	ARPKD
8	Pregnancy terminated	Not done	ARPKD	–	ARPKD
9	Pregnancy terminated	Not done	DCD	–	DCD

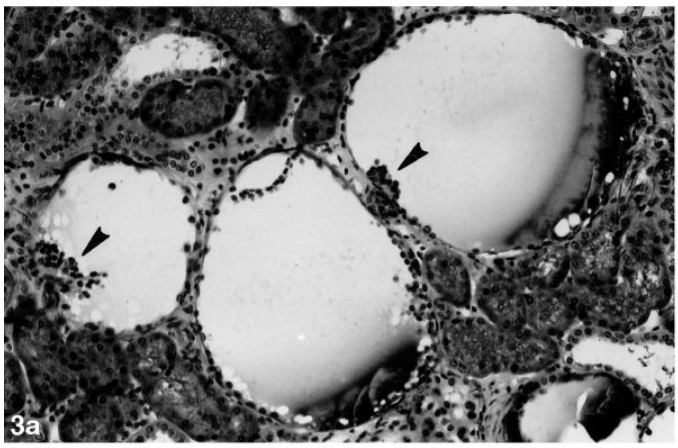
GCKD, Glomerulocystic kidney disease; ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; DCD, diffuse cystic dysplasia; GFR, glomerular filtration rate











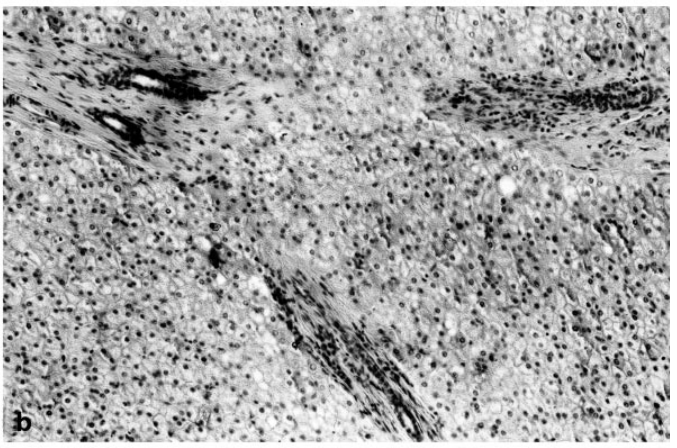


Fig. 1. Patient 1. **a** The kidney contains numerous glomerular cysts. The largest are in the inner cortex. Several (*arrowheads*) contain small glomerular tufts. The smaller cysts in the mid-cortex more often contain glomerular tufts. Rudimentary medullary development (*arrows*) accompanies the relatively severe cortical cyst formation hematoxylin and eosin (H and E, ×15). **b** The hepatic portal areas are somewhat enlarged by fibrous tissue. Mild biliary dysgenesis is demonstrated by the increased number of ductular profiles in the plane of section (H and E, ×120)

Fig. 2. Patient 2. **a** The kidney cortex contains numerous glomerular cysts, many with compressed glomerular tufts. The degree of cortical cyst formation is not as severe as in the kidney of patient 1, and medullary development (not shown) is relatively normal (H and E, ×25). **b** The hepatic portal areas are slightly enlarged, with mild biliary dysgenesis in the form of increased biliary profiles (H and E, ×160)

Fig. 3. Patient 3. **a** The cortex contains clustered glomerular cysts with residual tufts (arrowheads) (H and E, ×120). **b** The portal areas are enlarged and fibrotic, almost bridging, with an increased number of compressed and pinched biliary ducts (H and E, ×100)

period with renal cystic disease. These previous siblings carried the diagnosis of ARPKD. Renal sonograms were not performed on the parents of either fetus.

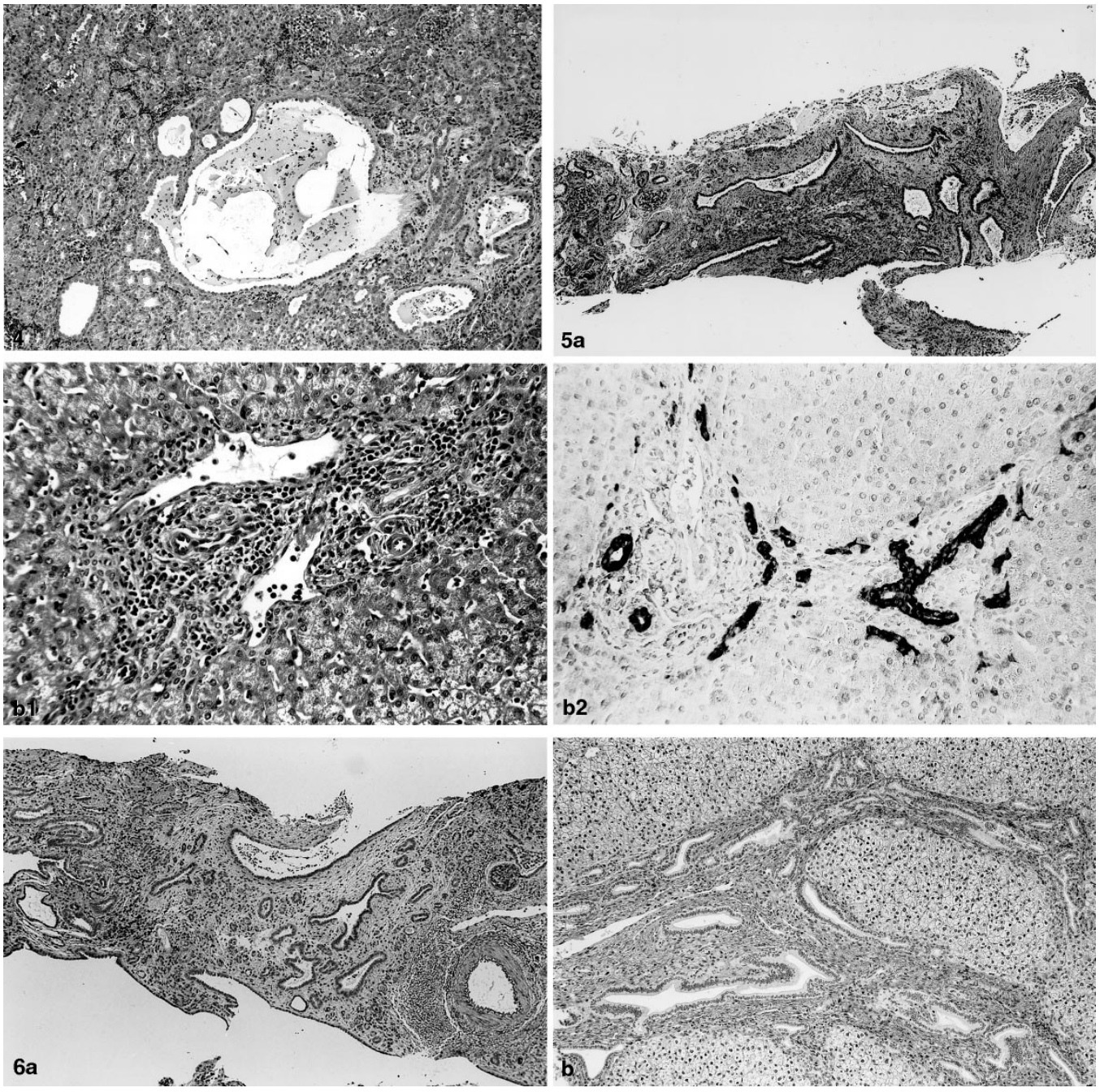


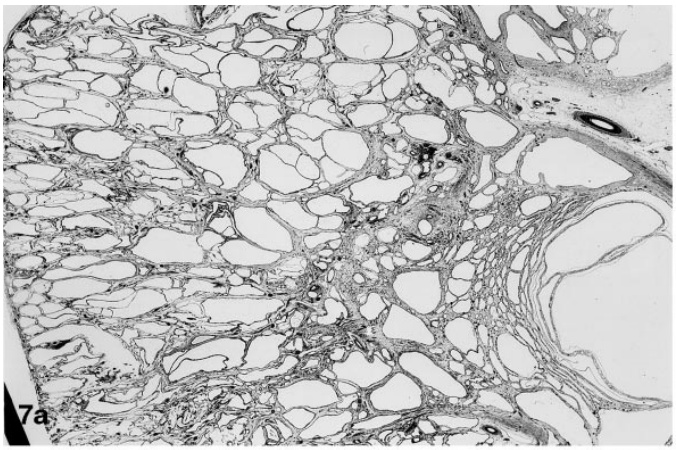
Fig. 4. Patient 4. The cortex contains seemingly isolated tubular cysts. This pattern is consistent with the early stages of classical autosomal dominant polycystic kidney disease (ADPKD) (H and E, ×60)

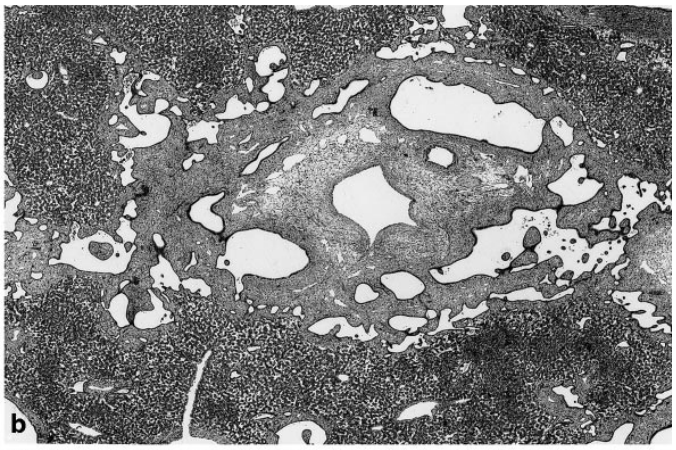
Fig. 5. Patient 5. **a** The medulla contains ectatic ducts and the interstitium is hypovascular, with a paucity of recurrent loops. These medullary changes are characteristic of autosomal recessive polycystic kidney disease (ARPKD) and are associated with small cortical cysts. There is also a moderate inflammatory cell infiltrate in the medullary interstitium and some collecting ducts contain purulent material (H and E, ×40). **b** Biliary dysgenesis, somewhat masked by inflammatory cell infiltration of the portal areas (**b1**), is more clearly shown by staining of the biliary ducts for cytokeratin (**b2**) (**b1**, H and E, ×190; **b2**, immunostaining for AE1/3 cytokeratin, ×160)

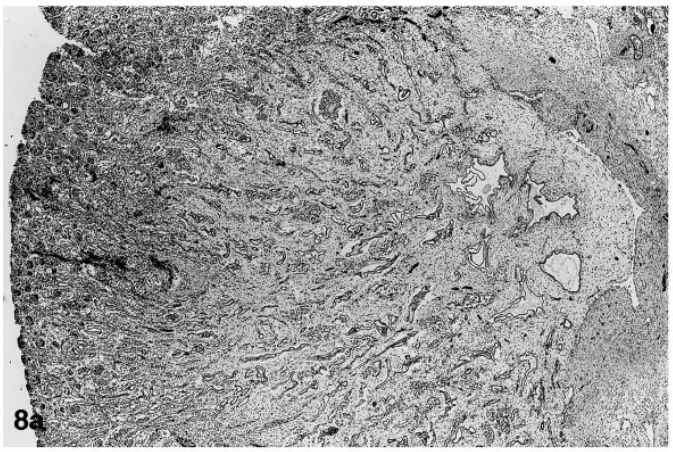
Fig. 6. Patient 6. **a** A section of outer medulla shows ductal ectasia with medullary interstitial hypovascularity and a paucity of recurrent loops. These changes are characteristic of ARPKD (H and E, ×50). **b** Hepatic biliary dysgenesis is associated with bridging portal fibrosis. This pattern represents a stage in the development of congenital hepatic fibrosis (H and E, ×60)

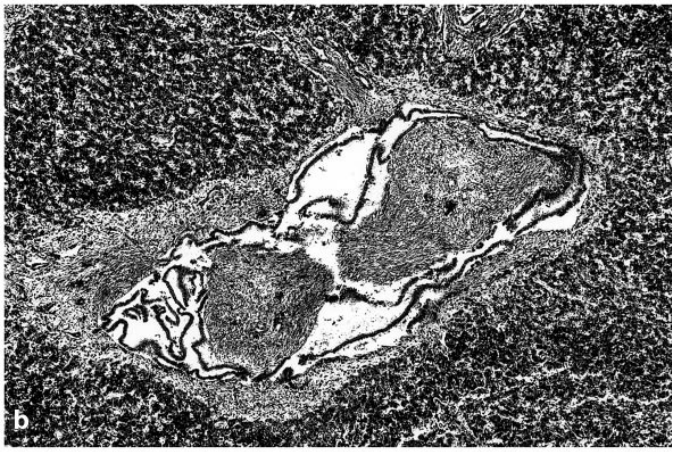
Clinical outcome

Further clinical and family data were obtained for several patients in the initial cohort. These data, as well as the histopathological analyses and genetic studies, are summarized in Table 2. Two years after patient 2's initial presentation, her 62-year-old paternal grandmother was noted as an incidental sonographic finding to have bilateral renal cystic disease consistent with ADPKD. By 3 years of age, the renal ultrasound in patient 3 was within normal limits in









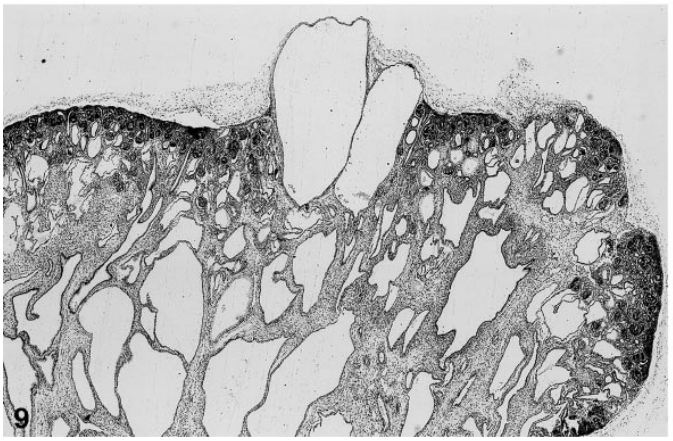


Fig. 7. Patient 7. **a** The kidney contains large, rounded, medullary ductal cysts and elongated cortical ductal cysts. The latter are arranged in an approximately radial fashion. The dilated cortical ducts traverse the entire thickness of widened cortex (H and E, ×6.0). **b** The liver contains enlarged portal areas, with numerous dilated biliary spaces that encircle the portal areas and extend into the adjacent hepatic lobules. These dilated biliary spaces freely intercommunicate and they may be a forerunner of non-obstructive intrahepatic biliary ectasia (Caroli syndrome) (H and E, ×12)

Fig. 8. Patient 8. **a** Severe fetal ARPKD is characterized by medullary ductal dilatation, with interstitial hypovascularity and paucity of recurrent loops. The dilated ducts, however, are partially collapsed due to fetal maceration (H and E, ×15). **b** Intrahepatic biliary dysgenesis is associated with portal fibrosis. The pattern of biliary ectasia in almost all portal areas throughout this random section indicates that the biliary structures are more likely flattened sacs than tubular bile ducts (H and E, ×40)

Fig. 9. Patient 9. The medullary ducts are dilated, somewhat like those in ARPKD, but the cortex is very thin. Reduced nephrogenesis is also evident in the discontinuity of the nephrogenic zone, which is interrupted by dilated ducts and fibrous tissue. The medulla does not form demarcated medullary pyramids, and the kidney is marked by poor caliceal development (H and E, ×15)

both size and echogenicity. In her twin, the right kidney was no longer visible, but the left kidney measured 6.6 cm and was slightly echogenic with obscuring of the corticomedullary junction. Re-review of the initial parental ultrasounds revealed that patient 3's then 37-year-old mother had a single 1-cm cyst in her right kidney. No further sonographic evaluation has been performed in this mother. The father of patient 5 was sonographically evaluated 6 months after her presentation and found to have enlarged, cystic kidneys consistent with ADPKD.

Histopathologic analyses

In three infants (patients 1, 2, and 3) histopathological analysis revealed glomerular cysts and minor tubular involvement (Figs. 1–3). In the neonatal autopsy specimen (patient 1), abnormal renal medullary development was evident with rudimentary medullary pyramids. Liver his-

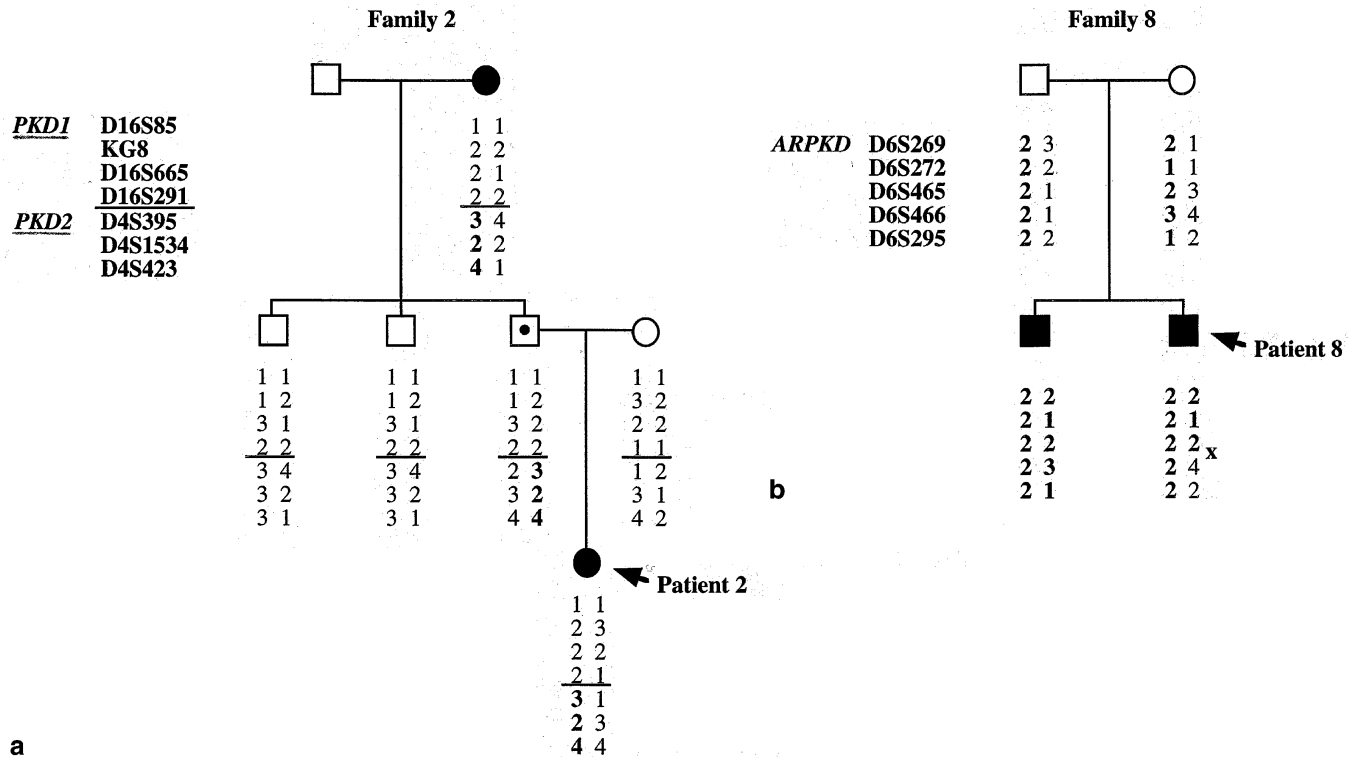


Fig. 10a,b. Haplotype analysis in two families with polycystic kidney disease. For each individual, the paternal haplotype is represented on the *left* and the maternal haplotype on the *right*. *Filled symbols* indicate affected individuals. The symbol with the central dot represents the presumably affected father of patient 2. **a** ADPKD family. Genotypes for the *PKD1* and *PKD2* interval markers are separated by a *bar* and listed below each individual's symbol. Alleles are displayed in the most likely haplotypes. The *PKD2* haplotype shared by the paternal grandmother, the father, and patient 2 is highlighted in bold. **b** ARPKD prenatal diagnosis. Genotypes for the ARPKD interval markers are listed below each individual's symbol.The disease-associated haplotypes are highlighted in bold. In patient 8, an apparent recombination event in the maternal haplotype is indicated by an *x*

topathology reveal biliary dysgenesis or "ductal plate malformation." This biliary lesion is characterized by enlarged portal areas containing elongated, proliferating bile ducts that often encircle the portal vein. In patient 1, the biliary abnormality, while not severe, was associated with increased portal fibrosis. This pattern of kidney and liver involvement has been described in neonates with either sporadic GCKD or infantile-onset ADPKD. Biopsies performed in patient 2 at 1 month of age and patient 3 at 2 years of age have a milder expression of the disease, but the glomerular and biliary abnormalities are qualitatively the same.

The percutaneous renal biopsy in patient 4 contained small clusters of tubular cysts located amidst otherwise normal glomerular and tubular structures (Fig. 4). The pattern is consistent with an early stage of ADPKD, although no family history of renal cystic disease was revealed either at the time of the patient's presentation or with follow-up questioning.

In patient 5, the renal histopathology was most notable for dilated cortical tubules and medullary ductal ectasia (Fig. 5a). The glomeruli generally had a mature appearance and there was no evidence of glomerular cysts. Moderate biliary dysgenesis was present in the liver (Fig. 5b1 and b2), but partially obscured by chronic cholangitis. In patient 6, sections from the percutaneous renal biopsy contained numerous ectatic collecting ducts surrounded by mildly to moderately fibrotic interstitial stroma (Fig. 6a). The dilated ducts were lined by an often hyperplastic cuboidal epithelia. The glomeruli had a fetal appearance, but no cystic dilatation of Bowman's space was evident. The bile ductules were increased in number and were most often seen in semicircular profiles around the portal vein (Fig. 6b). Moderate fibrosis was evident in the portal area. In both of these children, the renal histopathology was characteristic of the cystic lesion evident in older patients with ARPKD.

The autopsy in patient 7 revealed the classic features of neonatal ARPKD. Nephrogenesis was essentially completed. Dilated tubules were radially arrayed in the cortex in an orientation perpendicular to the renal capsule (Fig. 7a). These tubules often contained branch points, confirming their identification as collecting ducts. While the cortical collecting ducts were elongated, the medullary collecting ducts were very large and spherical. The liver contained severe biliary dysgenesis, with the ductules forming anastomosing channels around the peripheries of portal areas (Fig. 7b). These grossly dilated bile ductules may be a forerunner of Caroli syndrome.

The renal lesion in patient 8 was most remarkable for microcystic collecting ducts that ran radially from the medulla to the nephrogenic zone (Fig. 8a). Active nephrogenesis was evident and appeared to be proceeding normally. The bile ducts were increased in number and tortuous in their configuration (Fig. 8b). Moderate portal

tract fibrosis was present. In comparison, sections of the kidney from patient 9 revealed irregularly shaped ductal cysts. These cysts were largest near the renal hilum and smallest in the cortex. Active nephrogenesis was present. However, nephrogenesis appeared to have been diminished, as evidenced by the poorly differentiated medulla and very few well-developed nephrons in the cortex. The peripheral nephrogenic zone was repeatedly interrupted by dilated ducts and sometimes by fibrous tissue (Fig. 9). Mild-to-moderate biliary dysgenesis was also present (data not shown). While the medullary lesion is reminiscent of ARPKD, the renal histopathological features are distinctive and typical of Meckel syndrome. Consistent with this diagnosis, cervical rachischisis and an occipital defect were identified at autopsy, but polydactyly was not evident.

Genetic studies

Three families (patients 2, 5, and 8) were available for genetic analysis. The family of patient 2 was typed for markers of the *PKD1* and *PKD2* intervals and haplotypes were constructed (Fig. 10a). While the markers of the *PKD1* interval were not very informative in this family, clear haplotypes could be constructed with the *PKD2* interval markers. Patient 2 shares the same *PKD2* haplotype with her father and her affected paternal grandmother. These genetic data and the renal histopathology in patient 2 are consistent with ADPKD due to a mutation in *PKD2*. In this family, the disease expression appears to be highly variable, with an infantile presentation of ADPKD in patient 2 and asymptomatic ADPKD in her paternal grandmother. The father's renal status was assessed by ultrasound at the time of his daughter's initial evaluation and judged to be normal. Subsequent studies have not been performed.

In patient 5, the renal histopathology is most consistent with ARPKD. However, this diagnosis is confounded by the evidence of probable ADPKD in her father. The family was not informative for genetic linkage analysis because no other affected family members could be identified. While each of her parents have had children by other unions, patient 5 is the only child of this union. Paternity has been confirmed by a standard DNA testing protocol (UAB DNA Diagnostics Laboratory; data not shown) and there is no evidence of parental consanguinity.

In patient 8, histopathological review confirmed the diagnosis of ARPKD in the index case (data not shown) and the parents requested haplotype-based prenatal evaluation in a subsequent pregnancy. As shown in Fig. 10b, the fetus (patient 8) inherited the disease-associated paternal haplotype. However, a recombination event occurred in the critical ARPKD interval between *D6S465* and *D6S466* on the maternal chromosome. Given the significant probability that the fetus could be affected, the parents elected to terminate the pregnancy at 21 weeks' gestation. As discussed above, fetal pathological examination was consistent with ARPKD.

Discussion

We have presented a series of infants and fetuses with diffuse renal cystic diseases. As noted, the spectrum of diffuse renal cystic disease includes ARPKD, ADPKD, GCKD, and diffuse cystic dysplasia. The classic presentation of each disorder has been well described and the distinctions well characterized. However, as illustrated in our series, overlap in the clinical presentation and to some extent, in the histopathological features can complicate the differentiation of these disorders.

In our series, medical evaluation for each infant was sought following sonographic detection of enlarged, echogenic kidneys. The fetuses were brought to our attention because they had previous siblings who presented with diffuse renal cystic disease and carried the diagnosis of ARPKD.

Enlarged, echogenic kidneys most frequently occur in either recessive or dominant polycystic kidney disease [10, 11]. Massive, bilateral renal enlargement is typically recognized in ARPKD patients by sonography either in utero or at birth. Oligohydramnios is a common feature due to poor fetal urine output [12], but it rarely occurs before the 20th week of gestation [1, 13]. As a result of the oligohydramnios, affected infants develop the Potter sequence, a phenotype consisting of pulmonary hypoplasia, a characteristic facies, and deformities of the limbs [1, 14, 15]. In affected neonates, the clinical course is often characterized by respiratory insufficiency and death within the first few hours of life. Less severely affected infants usually survive, and their clinical course may be characterized by hypertension, progressive renal insufficiency, and the development of portal hypertension [11, 16, 17]. ARPKD has two invariant histopathological features: (1) dilatation of the renal collecting ducts and (2) biliary dysgenesis associated with portal tract fibrosis [18]. In neonates with severe disease, fusiform dilation of the cortical collecting ducts dominates the renal histopathological pattern, whereas in older children with milder disease, the predominant finding is medullary ductal ectasia.

The renal cystic lesions in patients 8, 7, and 6 represent the spectrum of ARPKD. In patient 8, both normal nephrogenesis and microcystic dilatation of the medullary collecting ducts were evident. Hyperechoic kidneys were identified prior to termination of the pregnancy at 21 weeks', but there was no associated oligohydramnios. In comparison, third-trimester fetal sonography in patient 7 revealed massively enlarged, hyperechoic kidneys and oligohydramnios.

The normal pattern of nephrogenesis proceeds in a centrifugal fashion, from the inner cortex to the periphery, and is completed by 34 weeks' gestation [19–21]. In ARPKD, nephrogenesis proceeds normally and the earliest abnormality involves the medullary ducts. Oligohydramnios occurs subsequently (usually $>$20–21 weeks' gestation). These observations suggest that in severe fetal ARPKD, medullary collecting duct dilatation occurs first and is successively followed by cortical collecting duct dilatation, increased renal echogenicity, and diminution of urine production.

In those who survive the perinatal period, like patient 6, the kidney size and extent of cystic involvement tend to be more limited [22]. These cystic changes are typically accompanied by progressive glomerular obsolescence, tubular atrophy, and interstitial fibrosis [23]. As a result, the gross contour of the kidney becomes more irregular and a reduction in kidney size may occur. Based on a longitudinal analysis, Lieberman et al. [24] reported that kidney size in ARPKD patients peaks at 1–2 years of age, then gradually declines and stabilizes by 4–5 years of age. Prominent medullary ectasia persists, and secondary cortical cyst formation may be the principal renal manifestation of ARPKD in older children. Given this disease progression, ARPKD presenting in older children may be difficult to distinguish from ADPKD.

Patient 5 may represent such a phenotypic overlap. Her renal histopathology was notable for cortical cysts and prominent medullary ductal ectasia. In addition, she had biliary dysgenesis. While this biliary lesion is an invariant finding in ARPKD, it also has been reported in ADPKD infants [25, 26]. Some might suggest that because ARPKD is distinctly uncommon in the African-American population, the diagnosis of ADPKD is more likely in this African-American child. However, this premise is not well established and, in our own series, patient 7 is an African-American with the classic features of neonatal ARPKD. Direct gene-based diagnosis is not yet available for ADPKD or ARPKD. In the absence of such direct analysis, parental and even grandparental screening is crucial for establishing the diagnosis of ADPKD in affected children. Follow-up parental sonography for 30% to 70% of ADPKD patients diagnosed in utero or in the 1st year of life identifies a previously undiagnosed affected parent [27]. In the case of patient 5, the identification of ADPKD in her father and confirmation of paternity stands in direct contrast to her renal histopathology. Resolution of the diagnostic dilemma for this patient must await direct, gene-based mutational analyses.

ADPKD diagnosed *in utero* or in the 1st year of life is reportedly associated with more severe renal cystic disease. While the majority of ADPKD infants survive, they tend to have more significant hypertension and a more rapid decline in renal function than their affected adult relatives [28]. In affected fetuses, dominant PKD is often associated with oligohydramnios, which may have an earlier onset than in recessive PKD. In fact, the renal developmental abnormality may occur earlier in gestation in ADPKD because the cortical cysts are associated with abnormal medullary differentiation. This lesion takes the form of small pyramids, poorly demarcated from the connective tissue of the renal sinus. The calices are very small, fornices almost absent. Collecting ducts are variably dilated, ectatic, and are surrounded by increased interstitium (see Fig. 1A and compare with Figs. 7A and 8A). Fibromuscular collars may be present. In comparison, the kidney in ARPKD is cystic without being abnormally differentiated [1].

If patient 5 does have ADPKD, her clinical disease has been more aggressive than that manifest in her father. In comparison, patient 4 presented at 5 months of age with a renal cystic lesion that is most consistent with ADPKD, and she has had a comparatively benign course. It remains unclear whether the renal cystic disease in patient 4 represents a spontaneous ADPKD mutation or delayed clinical onset in her transmitting parent. Recent estimates suggest that spontaneous mutations occur in fewer than 10% of ADPKD patients [29], but there are no specific estimates of the frequency in children. In comparison, ADPKD develops in less than 5% of adults older than 30 years of age who have a negative renal sonogram. Both parents of patient 4 were over 30 years of age when their initial renal sonograms were performed. While computed tomograhic scanning is more sensitive than sonography in detecting subtle changes of ADPKD [28], these studies have not been pursued in patient 4's parents.

It is important to note that sporadic GCKD and GCKD occurring in the context of familial ADPKD are clinically, sonographically, and histopathologically indistinguishable. The kidneys in both forms of GCKD often contain abnormally differentiated pyramids, a type of medullary dysplasia [30]. Both forms of GCKD are associated with biliary dysgenesis in about 10% of cases [30]. Patients 1 and 3 appear to have sporadic GCKD, but the late appearance of ADPKD in a parent cannot yet be ruled out. Of particular note, a single renal cyst was detected in the 37-year-old mother of patient 3, thus raising the odds that this mother and her twins have ADPKD. The apparent resolution of the sonographic abnormalities in patient 3 is an intriguing phenomenon, which in the absence of a repeat biopsy cannot be adequately explained.

ADPKD results from mutations in one of at least three distinct genetic loci, *PKD1*, *PKD2*, and *PKD3* [31–33]. *PKD1* has been mapped to chromosome 16p13.3 [31]. Approximately 85% of Caucasian families have the chromosome 16-linked form of the disease [34]. Of note, several groups have confirmed linkage to chromosome 16p markers in ADPKD families who have had affected fetuses and neonates [35–37]. With the recent isolation of the *PKD1* gene [4, 38, 39], direct mutational screening is on the horizon. At the present time however, such analyses remain in the research laboratory because of the genetic complexity of the *PKD1* locus. A second ADPKD gene, *PKD2*, has been localized to the long arm of chromosome 4 (4q13-4q23), and recently the mutant gene has been identified [5, 6, 40]. Affected individuals in these families appear to have a phenotype similar to that in *PKD1* families, but the age of onset of cystic disease, hypertension, and renal insufficiency is delayed [41, 42].

GCKD is a common manifestation of ADPKD in infants, as illustrated by patient 2. However, *PKD1* and *PKD2* haplotype analysis in her family yielded somewhat unexpected results. Mutations in *PKD2* account for less than 15% of ADPKD cases, and the *PKD2* patients studied to date have a relatively delayed clinical onset. It seems unlikely, therefore, that mutations in the *PKD2* gene would cause symptomatic ADPKD in children. Yet, patient 2 shares the same *PKD2* haplotype with her father and her affected paternal grandmother. While the predictive value of these data is limited by the fact that only two family members have ADPKD and the *PKD1* haplotypes are not very informative, direct analysis of the *PKD2* gene is now technically feasible. These molecular studies are in progress (S. Somlo, unpublished data). If a *PKD2* mutation is

confirmed, this finding would provide the first evidence that *PKD2* is involved in infantile-onset GCKD/ADPKD.

For the families of infants with renal cystic disease, the recurrence risk in subsequent pregnancies and the feasibility of prenatal detection can be pressing issues. Mendelian genetics dictates that for a dominant disorder each pregnancy has a 50% recurrence risk and for a recessive disorder, a 25% recurrence risk exists. The diagnosis of ADPKD in patient 2 allows more accurate counselling for her family about recurrence risk. If a *PKD2* mutation is confirmed in patient 2, these data could provide the basis for gene-based prenatal diagnosis in this family with widely variable disease expression. Recent surveys of ADPKD families indicate that only 4% would consider pregnancy termination if the fetus was affected [43], and thus genetic linkage analysis for *PKD1* or *PKD2* is not routinely performed for prenatal diagnosis in the United States or Europe [44]. It remains to be seen whether the prevailing attitude will change once gene-based diagnosis is available.

ARPKD, in comparison, is associated with a significantly worse prognosis, as an estimated 30%–50% of affected infants die in the perinatal period. Ultrasound-based prenatal evaluation has limited reliability due to the relative insensitivity of second-trimester fetal sonography [45–47]. The ARPKD locus has been mapped to 6p21-p12 and linkage analysis indicates that this disorder involves a single defective gene ([8]; Zerres and Guay-Woodford, unpublished data). These data have provided the basis for genetic linkage or haplotype-based prenatal diagnosis in at-risk families. The affected sibling of patient 8 had histopathogically proven ARPKD. Genetic linkage analysis revealed that patient 8 inherited the same paternal haplotype as his affected sibling, but had a recombination event in the critical ARPKD interval of his maternally derived haplotype. Given the substantial risk that this second fetus was affected, the parents opted to terminate the pregnancy. Histopathological analysis revealed that patient 8 had the classic features of fetal ARPKD.

It is important to stress that haplotype-based prenatal analysis is an indirect method whose accuracy depends fundamentally on the correct diagnosis in the proposed index case. For example, the previously affected sibling of patient 9 was reported to have ARPKD. The presence of an occipital defect in patient 9 raised questions for us about the accuracy of this previous diagnosis. The renal cystic lesion in patient 9, characterized by ductal cysts and abnormal renal differentiation, was more consistent with diffuse cystic dysplasia. The associated encephalocoele pointed to the diagnosis of Meckel syndrome. The principal locus for this disorder has been assigned to the long arm of chromosome 17 (17q21-q24) [48] and thus is separate and distinct from the ARPKD locus. Therefore, accurate phenotypic diagnosis in previously affected children is essential for reliable haplotype-based prenatal testing.

In summary, diffuse renal cystic disease presenting in neonates and infants has several possible etiologies. These various disorders are genetically distinct but share overlapping clinical, radiographic, and morphological features. Renal cysts may form as a consequence of a developmental abnormality, as in cystic dysplasia, or cyst formation may be superimposed on a template of initially normal renal differentiation, as in ARPKD. Family history, the presence or absence of syndromic features, and histopathological analysis can usually distinguish these disorders from one another. The clinician must be aware, however, that overlap in the clinical presentation, and to some extent the histopathological features, can complicate the diagnostic evaluation. In addition, parental cystic disease may not be detectable at the time of the child's presentation and there should be a low threshold for subsequent parental re-evaluation. Biliary dysgenesis is often associated with these renal cystic diseases, and therefore its presence cannot distinguish one specific disorder from another.

Recent genetic advances in PKD research are beginning to have a diagnostic impact. Molecular tools, such as genetic linkage analysis, are currently available for prenatal diagnosis of ARPKD and ADPKD in those families known to be at risk. In the near future, gene-based testing for ARPKD and ADPKD will be added to the clinician's diagnostic armamentarium. Direct genetic analysis will allow more precise prenatal diagnosis, should help resolve diagnostic dilemmas, and potentially, may provide prognostic information for affected children.

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