Diagnostic Approach in Autosomal Dominant Polycystic Kidney Disease

York Pei

Divisions of Nephrology and Genomic Medicine Department of Medicine, University Health Network and University of Toronto, Toronto, Ontario, Canada

Autosomal dominant polycystic kidney disease (ADPKD) is the most common Mendelian disorder of the kidney and affects all racial groups worldwide. It is characterized by focal development of renal and extrarenal cysts in an age-dependent manner. Typically, only a few renal cysts are detected in most affected individuals before 30 yr of age. However, by the fifth decade of life, hundreds to thousands of renal cysts will be found in the majority of patients. ADPKD is genetically heterogeneous. Mutations of two genes, *PKD1* and *PKD2*, account for approximately 85 and 15% of cases, respectively. Although the clinical manifestations of these two genotypes overlap completely, patients with PKD1 have much more severe renal disease compared with those with PKD2, as evidenced by their ESRD occurring approximately 15 yr earlier. Renal ultrasonography commonly is used for the assessment of ADPKD, and age-dependent ultrasound diagnostic criteria with high sensitivity and specificity have been established for individuals who are born with 50% risk for PKD1. Although these diagnostic criteria are used widely for genetic counseling and for the evaluation of at-risk individuals as living-related kidney donors to their affected relatives, their application to individuals who are at risk for PKD2 or have undefined genotype needs to be refined further. Molecular genetic testing is available for ADPKD and may be useful for evaluation of at-risk individuals with equivocal imaging results, younger at-risk individuals as a living-related kidney donor, and individuals with atypical or *de novo* renal cystic disease.

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Autosomal dominant polycystic kidney disease (ADPKD; MIM 173900) is the most common Mendelian disorder of the kidney and affects all racial groups worldwide, with a frequency of 1:500 to 1000 (1–4). It is characterized by focal and sporadic development of renal and extrarenal cysts in an age-dependent manner. Typically, only a few renal cysts are detected in most affected individuals before 30 yr of age. However, by the fifth decade of life, hundreds to thousands of renal cysts will be found in the majority of patients. In patients with established disease, their enlarged kidneys can each measure up to 40 cm in length (compared with 10 to 12 cm in normal) and weigh up to 8 kg (compared with 400 to 500 g in normal) (1). The degree of kidney enlargement, in turn, correlates with complications such as pain, hematuria, hypertension, and renal insufficiency. Overall, ADPKD accounts for 5% of ESRD and represents a major health care burden in developed countries (5). It also is associated with significant comorbidities from its extrarenal complications, such as valvular cardiac defects, colonic diverticulosis, inguinal hernias, and intracranial arterial aneurysms (1).

ADPKD is genetically heterogeneous. Mutations of two genes, *PKD1* (MIM 601313) and *PKD2* (MIM 173910), respectively, account for approximately 85 and 15% of cases in the white population (6,7). The existence of a rare third gene for ADPKD has been suggested by the reports of several families who are unlinked to both known gene loci (8–12). However, recent confirmation of these findings has been lacking, and one such unlinked family actually was found to have bilineal inheritance of heterozygous *PKD1* and *PKD2* mutations (13). Polycystin 1 and 2, the gene products of *PKD1* and *PKD2*, are plasma membrane proteins and components of a novel multifunctional signaling pathway (14,15). Polycystin 1 is predicted to be a receptor of unknown function and may be involved in cell–cell and/or cell–matrix interaction. By contrast, polycystin 2 functions as a nonselective cation channel. Both proteins interact *in vitro* through their cytoplasmic region and transmit fluid flow–mediated mechanosensation through the primary cilium in renal epithelium (16). Disruption of polycystin signaling in ADPKD may cause cyst formation owing to the inability of tubular epithelial cells to sense mechanical cues that normally regulate tissue morphogenesis (15–17).

Renal disease progression is highly variable in ADPKD, with the onset of ESRD ranging from childhood to old age (1,2). Recent studies have documented that bilineal inheritance of heterozygous *PKD1* and *PKD2* mutations or genomic deletion of *PKD1* and *TSC2* can result in earlier and more severe renal disease in a small number of patients (13,18,19). For the majority of patients, however, the gene locus confers a major effect on renal disease severity: Patients with PKD1 developed ESRD approximately 15 yr earlier than did patients with PKD2 (20,21). In addition, a gender effect on renal survival (*i.e.*, ab-

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Address correspondence to: Dr. York Pei, 8N838, 585 University Avenue, University Health Network, Toronto, Ontario, Canada M5G2N2. Phone: 416-340-4257; Fax: 416-340-4999; E-mail: york.pei@uhn.on.ca

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sence of ESRD) that favors female patients is evident in PKD2 but not PKD1 (21–23). By contrast, a weak allelic effect (based on the 5′ *versus* 3′ location of the germline mutations) on renal disease progression may exist for PKD1 but not PKD2 (23). Last, both genetic and environmental factors can significantly modify the renal disease severity in ADPKD (24–26). At the level of individual patients, therefore, it is not reliable to predict the underlying genotype on the basis of renal disease severity.

Challenging Diagnostic Settings in ADPKD

The diagnosis of overt ADPKD generally is straightforward (1,2). Affected patients typically present with enlarged kidneys with multiple cysts bilaterally and a positive family history consistent with autosomal dominant inheritance. Additional clinical findings that are helpful for the diagnosis include the absence of symptoms and signs that are suggestive of other syndromic forms of renal cystic disease (see below and Table 1) and the presence extrarenal cysts, inguinal hernias, and cardiac valve abnormality such as mitral valve prolapse. However, ADPKD occasionally will present very early, within the first years of life, and may be confused with autosomal recessive polycystic kidney disease (ARPKD) and renal cystic dysplasia (27,28). In this setting, the presence of a positive family history and larger renal cyst size will help to differentiate ADPKD from ARPKD, and the presence of malformations and ureteral obstruction will help to differentiate renal cystic dysplasia from ADPKD. However, a family history may be absent in 20 to 40% of new patients in whom the diagnosis of ADPKD first is suspected from imaging studies that are performed to evaluate otherwise unexplained hematuria, abdominal mass, flank pain, or renal insufficiency (1,2). In these cases, the finding of ADPKD can be due to a *de novo* mutation or, more likely, ascertainment of a small PKD2 family with mild renal disease, which often is underdiagnosed (4,21). In the latter instance, ultrasound examination of both parents may demonstrate multiple renal cysts in one parent. In the case in which one or both parents are deceased, review of autopsy reports (if available) also may be helpful. Last, individuals who are born with 50% risk for ADPKD often are being evaluated as potential living-related kidney donors to their affected relatives. The issue in this case becomes one of disease exclusion, which can be diffi-

Table 1. Differential diagnosis of ADPKD[a]

Renal Cystic Disorder	Prevalence	Clinical Findings Not Found in ADPKD
Syndromic		
tuberous sclerosis	approximately 1:10,000	Autosomal dominant inheritance. Skin lesions (facial angiofibromas, periungual fibroma, hypomelanotic macules, Shagreen patch); retinal hamartomas; seizures; mental retardation; cortical tuber; subependymal giant cell astrocytoma; cardiac rhabdomyoma; lymphangioleiomyomatosis; renal angiomyolipoma. Contiguous deletion of *PKD1* and *TSC2* results in severe polycystic kidney disease in infancy or early childhood with ESRD typically occurring in the first two decades of life.
Von Hippel-Lindau syndrome	approximately 1:50,000	Autosomal dominant inheritance. Central nervous system and retinal hemangioblastoma; pancreatic cysts; pheochromocytoma; renal cell carcinoma in 25 to 45% of patients; papillary cystadenoma of epididymis.
medullary sponge kidney	approximately 1:5000	Familial clustering uncommon. Medullary nephrocalcinosis; 'paintbrush' appearance of renal papillae on intravenous pyelogram.
orofaciodigital syndrome	very rare	X-linked dominant inheritance. Lethal in affected male individuals; oral anomalies (hyperplastic frenula, cleft tongue, cleft palate or lip, and malposed teeth); facial anomalies (broad nasal root with hypoplasia of nasal alae and malar bones), and digital anomalies.
Nonsyndromic		
simple renal cysts	common	Rare under 30 yr but increase in number with age.
acquired renal cystic disease	common	Chronic renal insufficiency or ESRD with multiple renal cysts associated with normal-sized or small kidneys.

[a]ADPKD, autosomal dominant polycystic kidney disease.

cult in younger individuals using an imaging-based approach (see next).

Differential Diagnosis of ADPKD

Renal cysts can be a manifestation of both syndromic and nonsyndromic disorders other than ADPKD (Table 1) (28). A careful review of the history and clinical and radiologic examination may reveal clinical features of these disorders that are atypical of ADPKD. For example, tuberous sclerosis (TSC) frequently is associated with characteristic skin lesions, and the coexistence of renal cysts with angiomyolipomas is pathognomonic of this disorder (29). However, these findings may be absent at the time of diagnosis of the renal disease. Moreover, a rare syndrome has been reported to involve the deletion of both *PKD1* and *TSC2*, which lie in close proximity on chromosome 16p13.3 (18,19). Patients with this syndrome typically present with bilaterally enlarged polycystic kidneys during infancy, with progression to ESRD by their teenage years. A family history of renal cystic disorder may not be evident in this syndrome because many of the cases represent *de novo* mutations. Clinical features that are suggestive of TSC also may be absent in approximately 30% of cases, which may be diagnosed mistakenly as ADPKD (19). By contrast, the presence of retinal and central nervous system hemangioblastomas, multiple renal cell carcinomas, pheochromocytoma, papillary cystadenomas of the epididymis, or multiple pancreatic cysts should raise the suspicion for the diagnosis of Von Hippel-Lindau syndrome (30).

Simple renal cysts and acquired renal cystic disease are two common causes of nonsyndromic cystic disorders that may be confused with ADPKD (31–33). Simple renal cysts are common and increase with age in the general population. The prevalence of one or more simple renal cysts has been estimated to be 0 to 0.2% in individuals who are younger than 30 yr, approximately 2% in those who are aged between 30 and 49 yr, 11.5% in those who are aged between 50 and 70 yr, and 22% in those who are older than 70 yr (31,32). However, the stringent criteria that are used for ultrasound diagnosis of ADPKD make it very unlikely that simple cysts may be confused as ADPKD (see next) (34). Acquired renal cystic disease is a common disorder that is observed in 7% of patients with chronic kidney disease and in approximately 20% of patients with ESRD. Despite the presence of multiple renal cysts bilaterally with varying degree of renal insufficiency, the size of the kidneys in this disorder is either normal or small (33). By contrast, the presence of chronic kidney insufficiency in ADPKD generally denotes advanced disease and typically is associated with large polycystic kidneys that are several times the normal size.

Pretest Probability of ADPKD in Individuals Who Seek Diagnostic Testing

The pretest probability (prevalence) of disease in the study cohort has a significant impact on the performance of any diagnostic test (35). Whereas individuals who have an affected first-degree relative have a 50% probability of ADPKD at birth, the risk for those who present later for diagnostic testing is not constant but diminishes with age. To illustrate this concept, Figure 1A shows a distribution plot on the relative proportions of individuals who are affected but have clinically undiagnosed disease, individuals who are affected and have a clinical diagnosis, and at-risk but unaffected individuals within a mixed PKD1 and PKD2 cohort over time. With increasing age, more affected individuals will have received a clinical diagnosis because of their symptoms (*e.g.*, hypertension, hematuria, flank pain from cyst rupture or passage of stone, urinary tract infection), whereas those who seek diagnostic testing will be those who are affected but have clinically undiagnosed disease or are at risk but are unaffected. The pretest probability of disease at any time point can be estimated by the ratio of those who are affected but have clinically undiagnosed disease to the total pool of at-risk individuals who seek diagnostic testing. As shown by Figure 1B, the pretest probability of disease at birth is 50% for individuals who are at risk for PKD1 and PKD2 but

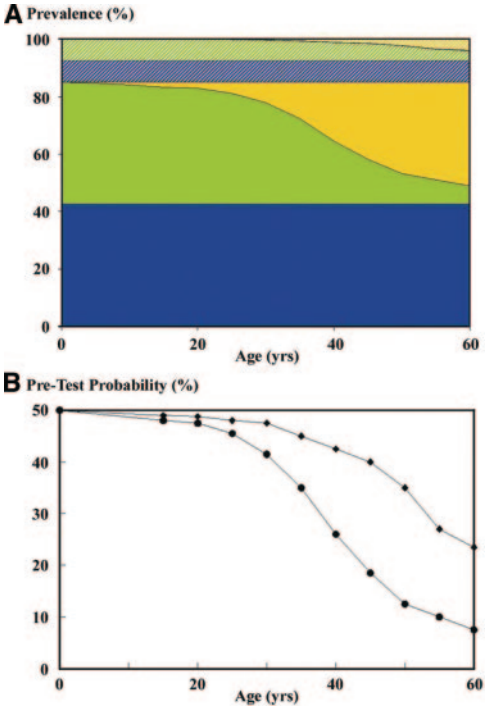

Figure 1. The pretest probability of disease decreases with age in individuals who are born with 50% risk for autosomal dominant polycystic kidney disease (ADPKD) and differs between PKD1 and PKD2. (A) The prevalence of PKD1 (solid color) and PKD2 (striped color) are assumed to be 85 and 15%, respectively. At birth, the proportion of individuals who are affected but have clinically undiagnosed disease (green) is equal to that of at-risk but unaffected individuals (blue). With increasing age, the proportion of those who are affected but have clinically undiagnosed disease diminishes as a result of increasing proportion of affected individuals' receiving a clinical diagnosis (yellow). However, a higher proportion of affected individuals with PKD1 will receive a clinical diagnosis (solid yellow) compared with affected individuals with PKD2 (striped yellow), because the former has much more severe renal disease. (B) The pretest probability of disease among individuals who are born with 50% risk for PKD1 (●) and PKD2 (♦) diverges with increasing age. Data from reference (21).

begins to diverge beyond the second decade of life because PKD1 is a more severe and symptomatic disease (21). These risk estimates have been incorporated appropriately in the age-dependent ultrasound diagnostic criteria for PKD1 to derive positive and negative predictive values (see next and Table 2) (34). A corollary of this is that these predictive values, which are derived from at-risk individuals with a positive family history, should not be used for patients with *de novo* ADPKD, because their pretest disease risk is very much lower (approximately 0.1 to 0.2%).

Renal Imaging in ADPKD

Renal ultrasonography is used widely for presymptomatic screening of at-risk individuals and for evaluation of potential living-related kidney donors from families with ADPKD. Using DNA linkage results as the "gold standard" for disease assignment, age-dependent ultrasonographic diagnostic criteria with high positive and negative predictive values have been derived for individuals who are born with 50% risk from families with PKD1 (Table 2) (34). Among at-risk individuals who are between 15 and 29 yr of age, the presence of at least two renal cysts (unilateral or bilateral) is sufficient for diagnosis. This is because simple renal cysts are rare in this age group, making the finding of any renal cyst highly specific for ADPKD. By contrast, more stringent criteria are required for the older age groups because of increasing prevalence of simple renal cysts. Among at-risk individuals who are aged 30 to 59 yr and 60 yr or older, the presence of at least two cysts in each kidney and at least four cysts in each kidney, respectively, are required for the diagnosis. Conversely, for individuals who are aged 30 to 59 yr, the absence of at least two cysts in each kidney, which is associated with a negative predictive value of 100%, can be used for disease exclusion. In general, a negative renal scan in individuals who are younger than 30 yr should not be used for disease exclusion because there is a false-negative rate of approximately 4% in this age group.

Although these diagnostic criteria for PKD1 are used widely for genetic counseling and for the evaluation of at-risk individuals as living-related kidney donors to their affected relatives, the underlying genotype of most test subjects seldom is known in the clinic setting. Given that PKD2 is a much milder disease (20,21), the validity of these diagnostic criteria for at risk individuals of unknown genotype has not been well defined. For addressing this issue, a multicenter study recently compared the performance of various ultrasonographic diagnostic criteria in a large cohort of at-risk individuals with PKD1 and PKD2 using their genotype as the gold standard for disease assignment (36). This study showed that the current diagnostic criteria that are used for PKD1 maintained high specificity but suffered from reduced sensitivity (increased false-negative rate) in PKD2. Therefore, the commonly used PKD1 criteria are expected to perform well for diagnosing PKD2. However, further refinement of the current criterion is required for exclusion of PKD2. By simulations, this study also evaluated a number of diagnostic criteria using replicates of patients with PKD1 and PKD2 to mimic the case mix that is seen in clinical practice. From this study, it is expected that a set of refined diagnostic criteria with high sensitivity and specificity will be available in the near future for the evaluation of at-risk individuals from families of undefined genotype.

Both computed tomography (CT) scan and magnetic resonance imaging (MRI), with and without contrast enhancement, have been used for diagnosis of ADPKD, generally in cases in which ultrasonography is equivocal (2,37). Both of these techniques, with improved resolution, can detect cysts of smaller size than ultrasonography. The major disadvantages of CT scan include the risk for exposure to radiation and radiocontrast that can be associated with a small chance of serious allergic reactions and nephrotoxicity in patients with renal insufficiency. By comparison, MRI, with gadolinium as a contrast agent, has little to no renal toxicity. This agent provides the same information as iodinated compounds do with respect to tubular function and parenchymal volume. Heavy-weighted T2 images also permit the detection of cysts of only 2 to 3 mm in diameter with great certainty (2). Its safety and enhanced sensitivity thus make it a very promising imaging modality for ADPKD (37). However, MRI likely will detect both small, simple cysts and small cysts that arise from ADPKD. Therefore, until its diagnostic utility has been evaluated formally as in ultrasonography, it should not be used as the initial imaging modality for diagnosis of ADPKD.

Molecular Genetic Testing in ADPKD

The identification of *PKD1* and *PKD2* followed by detailed characterization of their genomic structures has provided all of the essential reagents that are required for molecular diagnosis of ADPKD (38–40). Both DNA linkage analysis and gene-based mutation screening can be used for this purpose. As discussed previously, presymptomatic diagnosis of ADPKD typically is performed by renal ultrasonography. However, there is a role

Table 2. Performance characteristics of ultrasound diagnostic criteria for individuals who are born with 50% risk for PKD1[a]

Age Group (yr)	Diagnostic Criterion	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
15 to 29	At least two renal cysts (unilateral or bilateral)	approximately 96	100	approximately 97	100
30 to 59	At least two cysts in each kidney	100	100	100	100
60 or older	Four or more cysts in each kidney	100	100	100	100

[a]Data derived from reference (34). NPV, negative predictive value; PPV, positive predictive value.

for molecular diagnosis, especially in patients with equivocal imaging results, in those with a negative family history and in cases in which younger at-risk individuals with a negative ultrasound study are being evaluated as potential living-related kidney donor.

DNA Linkage Analysis

DNA linkage analysis requires both the genotype and the clinical information from multiple affected and unaffected family members, as well as from the at-risk individual being tested (13,34). By typing multiple polymorphic DNA markers that flank the *PKD1* and *PKD2* loci in the study subjects, linkage analysis seeks to identify a segment of the chromosome at either the *PKD1* or the *PKD2* locus that completely co-segregates with the disease. For clinical testing, Bayesian statistical algorithms are used to provide a probability estimate of linkage of the family to a disease locus. When the flanking polymorphic markers are informative, the prediction of disease in an at-risk individual can be highly accurate with an error of $<1\%$. The major advantage of this method is that when a large family with multiple affected members is available, it almost always is possible to determine whether the test subject is an obligate disease carrier. The identification of a pathogenic mutation, which can be challenging especially in PKD1, is not required. However, the need to obtain blood samples and clinical information from multiple individuals and the inconclusive findings of this method in small families are two main disadvantages.

Gene-Based Mutation Screening

PKD1 is a large gene with an approximately 14-kb mRNA transcript encoded by 46 exons. It is embedded in a complex genomic region, with the 5′ end of *PKD1*, including exons 1 to 33, reiterated at least six times more proximally on the same chromosome (38,39). Only approximately 2% of sequence divergence exists between *PKD1* and the *PKD1*-like pseudogenes, complicating specific amplification of the disease gene. By contrast, *PKD2* is a single-copy gene with an approximately 3-kb mRNA transcript encoded by 15 exons (40). Despite the challenges that are imposed by the genomic complexity of *PKD1*, mutation screening of the entire *PKD1* and *PKD2* coding sequences and their splice junctions from DNA templates now have been developed, allowing for comprehensive screening of these genes (41,42). To date, approximately 200 different *PKD1* and >50 *PKD2* mutations have been reported, with most of them predicted to truncate the mutant protein (as a result of frameshift deletions and insertions, nonsense mutations, or splice defects) (41–44). Most mutations are unique to a single family and scattered throughout these genes with no clear "hot spots" (41,44). Therefore, exon-by-exon screening of these genes is required to ensure a high sensitivity in detecting disease-causing mutations.

To define the sensitivity of the gene-based diagnosis of ADPKD, mutation screening of both *PKD1* and *PKD2* by denaturing HPLC followed by sequencing of PCR fragments with altered elution profiles was performed in a recent study of 45 patients with genetically uncharacterized ADPKD (42). In general, there was a high rate of missense mutations detected in *PKD1*, with an average of five per patient. By contrast, only two *PKD2* polymorphisms were detected in the entire patient cohort. Despite comprehensive screening of both genes, protein-truncating mutations in *PKD1* and *PKD2* was found in 26 patients and three patients, respectively. Therefore, the overall sensitivity for detecting a definitive mutation was 64% (29 of 45). The reasons for this relatively low mutation detection rate in this patient cohort are unclear. It is possible that some of the missense mutations identified in fact may be pathogenic. However, in the absence of a valid functional assay, it is difficult to differentiate the disease-causing missense mutations from benign polymorphisms. In addition, mutations in the gene promoter region, which may affect gene expression, have not been screened. Gene-based diagnosis of ADPKD is available commercially (Athena Diagnostics, Worcester, MA; http://www.athenadiagnostics.com). The main advantage of this test is that only a blood sample from the test subject is required. However, it is expensive, and although the sensitivity data for detecting a definitive mutation have not been published by the company, a definitive mutation likely is found in no more than approximately two thirds of the test subjects. Therefore, this screen is useful only when it is positive.

Diagnostic Algorithm for ADPKD

Figure 2 summarizes my personal approach in working up a patient who is suspected to have ADPKD. The first key point is to establish whether there is at least one affected first-degree relative, because the presence of a positive family history will greatly increase the pretest probability of ADPKD by two orders of magnitude from that of the population risk. Providing that there are no unusual clinical features in the test subject, age-specific renal ultrasound diagnostic criteria (Table 2) can be used to confirm the diagnosis in most instances. However, the presence of early and severe renal disease should raise the suspicion of PKD1-TSC2 deletion syndrome (18,19). Similarly, the presence of renal (*e.g.*, multiple angiomyolipomas, renal cell carcinomas) or extrarenal clinical findings (*e.g.*, facial and periungual fibromas, multiple pancreatic cysts) that are atypical of ADPKD also should raise the suspicion of other inherited renal cystic syndromes (Table 1). Patients with these unusual clinical features will need further evaluation, including additional imaging, referral for medical genetic consultation, and

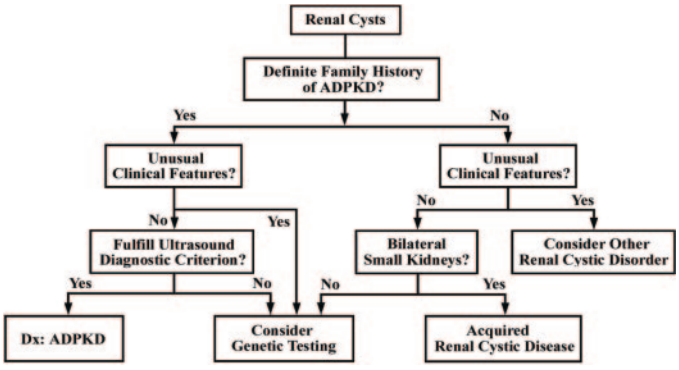

Figure 2. Diagnostic algorithm for ADPKD. See text for details.

molecular genetic testing. In the absence of a definitive family history, the diagnosis of ADPKD is much more challenging. In these cases, the finding of ADPKD can be due to a *de novo* mutation or, more likely, ascertainment of a small PKD2 family with mild renal disease, which often is underdiagnosed. In the latter instance, ultrasound examination may demonstrate multiple renal cysts in one parent. In the case in which one or both parents are deceased, review of autopsy reports (if available) also may be helpful. Conversely, the presence of atypical clinical features for ADPKD should raise the suspicion of other renal cystic disorders, and the presence of bilaterally small kidneys should raise the suspicion of acquired renal cystic disease (Table 1). Molecular genetic testing may be indicated for the evaluation of at-risk individuals with equivocal imaging results, younger at-risk individuals as a living-related kidney donor, and individuals with atypical or *de novo* renal cystic disease. In the future, the role of genetic testing in ADPKD may expand if effective disease-modifying drugs become available and the genotype of the patients influences their treatment decision (45,46).

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