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DNA Testing For Live Kidney Donors At Risk For Autosomal Dominant Polycystic Kidney Disease*

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by age-dependent growth of kidney cysts with end-stage renal disease developing in about 50% of affected individuals. Living donors from ADPKD families are at risk for developing ADPKD and may be excluded from renal donation if the diagnosis cannot be conclusively ruled out. Radiographic imaging may be adequate to screen for kidney cysts in most at-risk donors but may fail to identify affected individuals younger than 40 or older individuals from families with mild disease. In this paper we report a strategy that incorporates genetic testing in the evaluation of live kidney donors at risk for ADPKD whose disease status cannot be established with certainty on the basis of imaging studies alone. We show that DNA diagnostics can be used to enhance safe donation for certain living donor candidates at risk for ADPKD.

Keywords

living donation; polycystic kidney disease; genetic screening; molecular diagnostics; DNA testing

Autosomal dominant polycystic kidney disease (ADPKD) affects approximately 1/1000 individuals (1,2) and progresses to end stage renal disease (ESRD) in 50% by the sixth decade (3). ADPKD results from mutations in *PKD1* or *PKD2* (4). *PKD1* mutations account for 85% of cases while the remainder are caused by *PKD2* (5). The major distinction between the two is that *PKD1* mutations are associated with a more aggressive course, resulting in earlier onset ESRD (54.3 vs. 74.0 years) (6).

*Conflict of Interest Disclosure:

Under a licensing agreement between Athena Laboratories and the Johns Hopkins University, Dr. Watnick and her spouse are entitled to a share of royalty received by the University on sales of products described in this article. They have elected to donate their share of the royalty to the Polycystic Kidney Disease Research Foundation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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Many ADPKD patients are candidates for renal transplantation. Since deceased donor organs are in short supply, living related donation should be considered if ADPKD can be excluded in the prospective donor. In the majority of cases, a diagnosis of ADPKD can be determined via any of the available imaging modalities. Typical findings include bilaterally enlarged cystic kidneys frequently accompanied by extrarenal cystic disease. In some individuals with early stage disease, however, imaging may show few if any cysts. In order to address the question of how many cysts are sufficient to establish a diagnosis of ADPKD, Ravine et al. compared ultrasound imaging with genetic information in individuals at risk of inheriting *PKD1* (7). This analysis resulted in age-graded ultrasound criteria for ADPKD that have been broadly applied to all individuals with ADPKD. However, these criteria perform sub-optimally in patients at risk for *PKD2*, in whom cyst development is delayed and the disease course is often less severe (8,9). Pei et al recently extended this analysis to a population at risk for both *PKD1* and *PKD2*, devising ultrasound-based diagnostic criteria applicable to the situation typically encountered by clinicians where the PKD genotype is unknown (Table 1) (9). These revised criteria, however, may still yield equivocal results in the subset of patients younger than 40, where their negative predictive value ranges from 85–96%. In addition it is important to note that although the original Ravine criteria have been extrapolated to other imaging methods, analogous data for CT and MRI have not been collected. Because CT and MRI detect incidental renal cysts more frequently (10,11), false-positive diagnoses are possible when ultrasound criteria are extended to these modalities. The molecular diagnosis of ADPKD has improved substantially since identification of *PKD1* and *PKD2*. Both linkage testing and direct mutation analysis are commercially available for ADPKD (<http://www.genetests.com>). Linkage testing utilizes a panel of DNA markers flanking both PKD genes. By examining the distribution of these markers in affected and unaffected relatives, it is possible to ascertain the inheritance pattern of disease-carrying chromosomes (12). The major limitation of linkage analysis is that it requires the participation of multiple affected and unaffected family members. Moreover, relatives must have an established diagnosis for the results to be interpretable.

The commercially available direct DNA test for ADPKD involves sequencing of *PKD1* and *PKD2* (13,14). Several large series have demonstrated that 40–60% of tests in families with ADPKD yield diagnostic results, designated as “previously reported disease-associated mutations” or “predicted disease-associated mutations” (defined as previously unreported splice site, frameshift, or truncating mutations) (15,16). The primary reason for inconclusive results is that *PKD1* in particular is highly polymorphic (5) and direct sequencing may reveal only multiple amino acid substitutions. Although the pathogenicity of some missense mutations has been established (ADPKD mutation database: <http://pkdb.mayo.edu/>), the disease-causing potential of other variants (termed unknown amino acid change (UAA) in reporting forms) is uncertain (15).

We report herein a strategy of incorporating genetic testing in the evaluation of live kidney donors at risk for ADPKD. These cases serve to illustrate the utility and limitations of genetic testing for prospective donors with uncertain disease status. Two candidate donors younger than 25 years each sought live donation to a parent with ESRD secondary to ADPKD. An abdominal ultrasound in one and a CT scan in the other revealed normal-sized kidneys without kidney or liver cysts. Given the uncertainty of negative imaging in at-risk individuals younger than 30, genetic testing was pursued. Linkage testing was first considered, however insufficient numbers of affected relatives were available for either family. Therefore, we proceeded to mutation analysis. DNA sequencing of the recipient revealed a pathogenic *PKD1*-truncating mutation in each case (Table 2a, cases 1 and 2). Subsequent testing of the donor for the respective parental mutation was negative. Each donor candidate was considered unaffected by ADPKD and cleared for donation.

A third case involved a 45 year-old woman seeking to donate to her 36 year-old brother with ADPKD. Her MRI revealed 3 right renal cysts. Although the donor did not meet diagnostic criteria for ADPKD (2 cysts per kidney), she was reluctant to donate without DNA evidence that she was unaffected. As there was no other family history of ADPKD, linkage studies could not be performed. Mutation analysis of recipient blood revealed that the affected brother had a pathogenic frameshift mutation of *PKD1* (Table 2a, case 3). Subsequent testing of the donor for this mutation was negative, affirming that the patient was unaffected. She felt reassured and proceeded with donation.

In contrast to the cases described above, DNA testing failed to yield definitive results in the last donor candidate. A 35-year old male wished to donate to his 73 year-old father with long-standing nephrotic syndrome of unknown etiology and bilaterally enlarged cystic kidneys attributed to ADPKD (renal dimensions 14.2 and 15.3 cm in length by ultrasound). Donor CT showed three left renal cysts and none in the right kidney or liver. Although the donor did not meet criteria for *PKD1*-linked disease, the relatively late onset of ESRD in his father suggested possible *PKD2*-linked disease. Therefore, genetic testing was recommended. Because there was no other family history of ADPKD, linkage testing was not an option. Mutation analysis of the affected recipient's DNA sample revealed a number of polymorphisms and sequence variants of unknown significance (UAA), but no known pathogenic mutation in *PKD1* or *PKD2* (Table 2b). Since no diagnostic parental mutation was identified, DNA testing of the donor would yield similar sequence variants and would not clarify his disease status any further.

Based on our experience, we propose the following algorithm incorporating the use of DNA testing for the evaluation of donors at 50% risk of ADPKD inheritance (Figure 1).

First, potential donors should be counseled regarding the risks of establishing a presymptomatic diagnosis of ADPKD, including impact on future insurability. Initial evaluation should include renal imaging. Given that there are no established recommendations on the preferred initial imaging modality, the decision to initially screen with ultrasound, CT, or MRI depends on the clinical scenario. If there are several potential donor candidates, it may be less expensive to screen with ultrasound than with CT or MRI. A potential donor could be ruled out if there is radiographic evidence of ADPKD before proceeding further with donor evaluation. Alternatively, if there is only one donor candidate, initial screening with CT may be considered, as most centers already use CT for all donor candidates to define anatomy anyway. DNA testing should be pursued in candidates younger than 30 with fewer than 3 renal cysts, including those with absent renal cystic disease. Potential donors between 30–59 years of age with no renal cysts confirmed by CT may be allowed to donate but ADPKD should be excluded via genetic testing in those with a few non-diagnostic renal cysts (see Figure 1).

Linkage testing (Center for Genetic Testing at Saint Francis; Tulsa, OK) may be quicker and less expensive compared to DNA sequencing. As of 2008, linkage analysis for *PKD1* and *PKD2* costs approximately \$2500 with results expected in 3 weeks. In comparison, DNA sequencing (Athena Diagnostics, Inc.; Worcester, MA) costs between \$3,650 and \$4,580 for complete sequencing of the proband, and an additional \$620–770 for directed sequencing of each additional family member (costs vary depending on insurance plan and institutional contracts) with results returning in a minimum of 6 weeks. Therefore, when at least three affected family members are available, linkage analysis is recommended. The ADPKD status of each participant should be verified before ordering linkage studies. If the family structure does not permit linkage testing, direct mutation analysis is considered. The possibility of inconclusive DNA testing should be discussed with the family in advance. In order to minimize the expense of direct mutation analysis and to maximize the chance of

obtaining interpretable results, we recommend testing the recipient first. If a diagnostic mutation is identified, such as a “previously reported disease-associated mutation” or “predicted disease-associated mutation”, then limited, directed sequencing of the donor for the same mutation is performed (cases 1–3). If only indeterminate sequence variants such as unknown polymorphism(s) or unknown amino acid change(s) are identified in the recipient (case 4), we do not proceed with donor DNA sequencing. Depending on the clinical scenario, it may be necessary to defer donation. We note that directed donor DNA testing carries a small theoretical risk of overlooking a distinct *de novo* mutation. Assuming a 5% spontaneous mutation rate and disease prevalence of 1/1000, the risk is in the range of 1/20,000 (1,2).

The cases presented above illustrate the utility and complexity of genetic testing for ADPKD. The major limitations of molecular diagnostics include the requirement of family participation for linkage studies, the potential for inconclusive results with direct mutation analysis, and finally the cost. Although donors in the United States are not subject to expenses related to their evaluation, these fees eventually filter down to the transplant center and recipient insurance through the institutional organ acquisition charge. Undoubtedly, the use of genetic testing for transplant evaluation would add to the overall cost of transplantation. It is difficult to estimate the number of at-risk individuals in the United States who might require DNA testing as patient demographics and evaluation practices vary among transplant centers. Based on Organ Procurement and Transplantation Network (OPTN) data as of July, 2008, 2322 individuals with ADPKD were added to the deceased donor wait list while 667 patients with ADPKD received a living donor transplant in 2007 (accessed at <http://www.optn.org>). Of this latter group, 175 donors were either children or full siblings. There is no data, however, with respect to how many potential donors in these groups were turned down because of uncertain ADPKD status that might have been resolved with genetic testing. Because the long-term cost of dialysis exceeds that of transplantation, genetic testing would be cost-effective if its use led to an increase in the number of living donor transplants. To illustrate this, the cost of transplantation in the United States is approximately \$87,400 for the first transplant year (17) and an additional \$17,000 for each subsequent year with a functioning graft (18). In comparison, per person per year cost of dialysis is approximately \$69,000 (18). Assuming the average waiting time for a deceased donor kidney transplant is three years, the cost benefit of obtaining a living donor transplant early versus remaining on dialysis on the transplant waiting list for three years is approximately \$85,000 (roughly \$28,000/year). If linkage testing costs about \$2500 and DNA sequencing of a proband and one family member costs about \$5000, genetic testing would be cost-effective if at least one living donor transplant per year resulted from every 10 linkage tests or every 5 DNA sequencing tests performed.

Although the broad application of genetic testing for the evaluation of live donors at risk for ADPKD has not been systematically studied, its major benefit is likely to be in cases where disease status remains inconclusive despite the results of imaging studies. In these situations, the decision to proceed with genetic testing should be made after weighing the strengths and limitations of molecular diagnostics against the risks of kidney donation when ADPKD cannot be conclusively ruled out. Diagnostic algorithms provide an important framework for the evaluation of living donors and serve to guide the efficient utilization of available testing. When sufficient concern for ADPKD exists despite initial radiographic screening, molecular diagnostics can be an added tool in evaluating living donors from families with ADPKD.

Abbreviations

ADPKD	Autosomal dominant polycystic kidney disease
ESRD	End-stage renal disease
OPTN	Organ Procurement and Transplantation Network
UAA	Unknown amino acid change

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Figure 1.

***Equivocal imaging studies include the presence of any renal cysts by ultrasound, CT, or MRI in patients 30–39 years old and 2 or 3 cysts total in patients 40–59 years old. Individuals 40–59 years of age with ≤ 1 cyst may proceed with donation.

Table 1

Ultrasound Criteria for Diagnosis of ADPKD in Patients at 50% Risk of *PKD1* Inheritance (Adapted from Pei, et al. (9))

Age	Diagnostic Criteria by Ultrasound
<40	At least 3 renal cysts (unilateral or bilateral)
40–59	At least 2 renal cysts in each kidney
≥60	At least 4 renal cysts in each kidney

Table 2

Summary of DNA sequencing results. a) Pathogenic variants identified by DNA sequencing (cases 1–3). (Codon: a sequence of three nucleotides within DNA or messenger RNA that encodes for a specific amino acid; Frameshift mutation: a disruption of the reading frame used for translation from messenger RNA into amino acid by the insertion or deletion of a number of nucleotides that are a non-multiple of three into a group of codons; Stop codon: a tri-nucleotide sequence, or codon, within a messenger RNA which signals a termination of translation) b) Sequence variants identified by DNA sequencing (case 4). GenBank reference files: L33233 (*PKD1* cDNA position), AAC37576 (*PKD1* amino acid position), NM000297 (*PKD2* cDNA position) and NP00288 (*PKD2* amino acid position) (<http://www.ncbi.nlm.nih.gov/Web/Genbank/>).

a. Summary of pathogenic variants identified by DNA sequencing (cases 1–3)		
Case	DNA/Protein Variant	Variant Type
1	<i>PKD1</i> Exon 45: 12608delC, codon position 4133	Frameshift mutation
2	<i>PKD1</i> Exon 15: G3694A, T1161X	Stop codon
3	<i>PKD1</i> Exon 1: 364delC, codon position 51	Frameshift mutation

b. Sequence variants identified by DNA sequencing (case 4)			
DNA Variant	Codon Position	Amino Acid Change	Variant type
<i>PKD1</i> : C2387T	726	Leucine → Phenylalanine	Unknown amino acid change
<i>PKD1</i> : AIVS 9–4G	N/A	N/A	Unknown polymorphism
<i>PKD1</i> : TIVS27–13C	N/A	N/A	Unknown polymorphism
<i>PKD1</i> : AIVS30+54G	N/A	N/A	Unknown polymorphism
<i>PKD1</i> : T12838C	4209	None	Unknown polymorphism
<i>PKD1</i> : G13135A	3'UTR	None	Unknown polymorphism
<i>PKD1</i> : IVS9+28del7	N/A	N/A	Unknown polymorphism
<i>PKD1</i> : G2911A	900	None	Known polymorphism
<i>PKD1</i> : C2941T	910	None	Known polymorphism
<i>PKD1</i> : A4876C	1555	None	Known polymorphism
<i>PKD1</i> : T7376C	2389	None	Known polymorphism
<i>PKD1</i> : C7652C	2481	None	Known polymorphism
<i>PKD1</i> : AIVS24–17G	N/A	N/A	Known polymorphism
<i>PKD1</i> : G9406C	3065	None	Known polymorphism
<i>PKD1</i> : T9407C	3066	Phenylalanine → Leucine	Known polymorphism
<i>PKD1</i> : T9541C	3110	None	Known polymorphism
<i>PKD1</i> : C10743T	3511	Alanine → Valine	Known polymorphism
<i>PKD1</i> : A12341G	4044	Isoleucine → Valine	Known polymorphism
<i>PKD1</i> : C12384T	4058	Alanine → Valine	Known polymorphism
<i>PKD1</i> : A12484G	4091	None	Known polymorphism
<i>PKD1</i> : C12617T	4136	None	Known polymorphism
<i>PKD2</i> : G149C	28	Arginine → Proline	Known polymorphism