GUIDELINE RECOMMENDATIONS*

a. We recommend that the standard methodology for genetic diagnosis of autosomal dominant polycystic kidney disease is polymerase chain reaction (PCR) amplification (including long-range PCR for the first 33 exons of \(PKD1\)) followed by Sanger sequencing (1A) or next-generation sequencing where available (1D).

b. We suggest that individuals with a clinical diagnosis of autosomal dominant polycystic kidney disease in whom a mutation is not found by PCR amplification and sequencing have \(PKD1\) and \(PKD2\) analyzed for large genomic rearrangements (such as deletions) by quantitative fluorescent multiplex PCR or a custom-designed array comparative genomic hybridization (2B).

*Criteria used for recommendations and levels of evidence (1,2;A-D) are described in more detail in Tables 1 and 2 of Rangan G, Savige J. Introduction to the KHA-CARI Guidelines on ADPKD. Semin Nephrol.2015;35:521-3 in this issue.

UNGRADED SUGGESTIONS FOR CLINICAL CARE

- We suggest that next-generation sequencing technologies will soon be the standard methodology for genetic diagnosis in autosomal dominant polycystic kidney disease.

IMPLEMENTATION AND AUDIT

We suggest regular ongoing audit and/or review of the following:

1) The clinical indications for genetic testing of \(PKD1/PKD2\)

2) If/how a genetic test result has changed management and outcomes of patients.

BACKGROUND

Mutations in two genes are known to cause autosomal dominant polycystic kidney disease (ADPKD)—polycystic kidney disease 1 (\(PKD1\)) and polycystic kidney disease 2 (\(PKD2\)). For families in which mutations are...
found (noting that for 10% of patients who meet clinical diagnostic criteria for ADPKD, mutations in either *PKD1* or *PKD2* are not found with current testing methodologies). *PKD1* mutations account for 85% and *PKD2* mutations for 15% of cases.\(^3,4\) There is no evidence to support the presence of a third locus.\(^5\)

The *PKD1* gene is located on chromosome 16p13.3 and is large, consisting of 46 exons spanning 52 kb of genomic DNA and encoding a GC-rich transcript of 12,912 bp. The 5′ two thirds of the gene (exons 1-32) is duplicated within six pseudogenes (*PKD1P1-P6*) that share 97.7% sequence identity with *PKD1* but are not functional.\(^6,7\) Standard PCR and Sanger sequencing is thus ineffective across the *PKD1* locus. In contrast, the *PKD2* gene is located on chromosome 4q22.1, is single copy, and consists of a 2,904-bp open reading frame spread over 15 exons.

**SEARCH STRATEGY**

**Databases Searched**

Medical subject headings (MeSH) terms and text words for ADPKD were combined with the MeSH terms and text words for genetic testing. This was then combined with further searches using the MeSH and text words for diagnosis and genetic counseling, and animal studies were specifically excluded. The search was carried out in Ovid MEDLINE (1946 to June 2014), Embase (1974 to May 23, 2014), PsycINFO (1806 to June 2014), and Cochrane Database of Systematic Reviews and Cochrane Registry of Clinical Trials (inception to June 2014).

**Date of search:** June 2014.

**WHAT IS THE EVIDENCE?**

Supplementary evidence Tables are available online.

**Indications for Genetic Testing**

Genetic testing is not indicated in every patient as it remains expensive and results may be negative or uncertain, even in families in which the diagnosis is confirmed clinically. Situations in which genetic testing is appropriate include: (1) when a definitive diagnosis is required in young persons, such as a potential living-related donor in an affected family with equivocal imaging data; (2) to provide diagnostic clarity in patients with a negative family history of ADPKD in whom alternative causes of cystic kidney disease are considered; (3) in couples requesting genetic counseling and family planning advice, especially given the increased availability of preimplantation genetic diagnosis; (4) in cases of marked clinical discordance such as very early onset disease or very mild disease where knowledge of the mutation may provide an explanation or prognostic information. Genetic testing is best accessed through a Clinical Genetics unit in order to provide appropriate pretest counseling. The website www.genetics.edu.au currently provides a list of Clinical Genetics service providers in Australasia.

**Genetic Testing Methodologies**

Linkage analysis is an effective means of determining which of the two genes is involved in a family and can be used for cascade predictive testing; however, its usefulness is limited in smaller families or when fewer than three affected members are available for study. Some families will not have informative haplotype markers and linkage analysis cannot be used in such cases. Alternative molecular diagnostic techniques will therefore be required.

The current standard methodology for the molecular diagnosis of ADPKD is PCR amplification followed by Sanger sequencing of all exons. Sequencing of *PKD1* requires five long-range PCR amplicons covering the first 33 exons utilizing the few sequence sites unique to *PKD1* to provide templates for further PCR reactions. The remaining exons of *PKD1* and all 15 exons of *PKD2* are suitable for standard PCR amplification. Rossetti et al\(^8\) described the analysis of 45 unrelated probands using this amplification technique followed by denaturing high-performance liquid chromatography screening, and found 29 definite mutations. Audrezet et al\(^9\) analyzed 700 probands, including 528 with a family history of ADPKD, and demonstrated an overall mutation detection rate of 89%; 83.8% were *PKD1* mutations and 16.2% were *PKD2*. Those with a family history of ADPKD were more likely to have a definite pathogenic mutation found (69.1% versus 58.7% in those without a family history). The opposite was true for those in which only a probable mutation was found (21.6% with a family history and 28.5% in those without).

Next-generation sequencing, also referred to as massively parallel sequencing, has the potential to deliver faster diagnostic testing at considerably less cost than Sanger-based sequencing techniques. It may be performed on a number of different technology platforms, however, the underlying principle in each is similar. The target DNA is analyzed initially as vast numbers of short sequences, with these short sequences being read in parallel and matched to a reference sequence to allow the final target sequence to be built. Next-generation sequencing approaches to ADPKD diagnostics are in the early stages, but preliminary publications have demonstrated that this is likely to be the preferred technology in the future.
sequencing approach utilizing long-range PCR for initial target sequence amplification looking at 230 novel probands and 34 previously characterized patients found 2445 initial variants that were subsequently processed to 234 Sanger-verified variants. In their subset of clinically confirmed cases of ADPKD, they reached a confident molecular diagnosis in 63%. A group of 48 patients were analyzed with both technologies and this demonstrated that, when compared with long-range PCR and Sanger sequencing as the standard approach, their next-generation sequencing approach had 78% sensitivity, 100% specificity, and 60% accuracy. A recent study looking at 25 previously Sanger-sequenced probands demonstrated a sensitivity of 99.2% and a specificity of 99.9%. This study also found mutations in 3 of 24 probands in whom Sanger sequencing had failed to find a mutation. A novel approach by Trujillano et al in which long-range PCR was rendered unnecessary may provide a method that can be applied broadly. They correctly detected mutations in 35 of 36 of their proof-of-principle cohort, and then found 11 pathogenic mutations in 10 of 12 of their discovery cohort. Lastly, Eisenberger et al recently published a sequence capture–based next-generation sequencing approach that successfully detected mutations in 55 patients, including two patients with copy number variations. Their diagnostic algorithm allowed detection of mutations in PKD1, but by parallel analysis of captured sequence data, mutations in all other genes identified so far for polycystic kidney disease and related disorders, including PDK2, could be analyzed.

Variant Pathogenicity

There is significant allelic heterogeneity in PKD1 mutations with approximately 70% being unique; missense, nonsense, splice site, and small insertion-deletions may be found. Large genomic rearrangements, detectable by quantitative fluorescent multiplex-PCR and custom-designed array comparative genomic hybridization, are rare causes of ADPKD and represent less than 4% of cases.

It should be noted that the pathogenicity of variants may not always be clear, and caution should be taken in interpreting their clinical significance. For example, Rossetti et al found an average of 5.4 gene variations per proband in their initial screening study. Each variant requires analysis for pathogenicity. The Autosomal Dominant Polycystic Kidney Disease Mutation Database documents many variants that have been curated for pathogenicity, however, most families harbor unique changes and so further in silico analysis using such tools as Alamut (interactive biosoftware Rouen, France) are necessary. Very few mutations have been subjected to functional analysis. Segregation studies in other family members where available may also help clarify variants of unknown significance.

Genotype-Phenotype Correlations

There is evidence of genotype-phenotype correlation in ADPKD. Mutations in PKD1 cause more severe disease than PKD2, with end-stage kidney disease (ESKD) occurring approximately 20 years earlier, at an average age of 58 years versus 79 years. Additionally, carriers of truncating mutations in PKD1 reach ESKD approximately 12 years prior to those with missense mutations. Previous analyses suggested that the location of the mutation influences the age of ESKD, however, a more recent report has refuted this. It may be that a vascular phenotype is more likely if the mutations are located in distinct amino acids of the PKD1 gene. Hypomorphic PKD1 alleles that modify disease presentation have also been described.

The family in which the PKD1 gene was originally cloned also had tuberous sclerosis, and the co-occurrence of the two disorders was caused by a single chromosomal translocation with the breakpoint upstream of tuberous sclerosis 2 (TSC2) and within the PKD1 gene. The presentation of tuberous sclerosis complex and ADPKD in the same individual has now been documented in numerous families where a contiguous gene deletion encompasses both loci.

SUMMARY OF THE EVIDENCE

A number of large studies have shown mutations are detected in approximately 90% of ADPKD families when using PCR amplification and Sanger sequencing. Additional mutations such as large rearrangements are identified with appropriate technologies. These studies have shown that 85% of families have mutations in PKD1 and the remaining 15% in PKD2. Next-generation sequencing technologies show promise in delivering rapid diagnostic testing at considerably less cost than Sanger-based techniques and have been introduced in many laboratories for single-gene and gene panel tests; evidence suggests that it is equivalent in sensitivity, specificity, and accuracy, but further validation is required.

WHAT DO THE OTHER GUIDELINES SAY?

Kidney Disease Outcomes Quality Initiative: No recommendation.
UK Renal Association: No recommendation.
Canadian Society of Nephrology: No recommendation.
European Best Practice Guidelines: No recommendation.
International Guidelines: No recommendation.
Spanish Guidelines**: Working group on Inherited Kidney Disease with Spanish Society of Nephrology

1. Genetic testing for ADPKD is not recommended in routine clinical care when the clinical and imaging diagnosis is clear (ungraded statement).
2. Specific situations in which genetic testing for ADPKD may be valuable are related living-donor transplantation, uncertain de novo cases, very early onset disease, and preimplantation genetic diagnosis (D).
3. The method for genetic testing may be chosen based on the clinical presentation, the characteristics of the family, and the availability of genetic testing techniques (ungraded statement).

SUGGESTIONS FOR FUTURE RESEARCH

Further studies of next-generation sequencing technologies are warranted and likely to be forthcoming in the near future.

Confirmed pathogenic mutations should be submitted to an ADPKD mutation database.

The KDIGO 2014 Controversies Conference** identified gaps in knowledge and suggested a relevant research agenda on:

- Studies to better define the natural history of ADPKD in childhood, including different prognostic subgroups (ie, mutation screen and/or total kidney volume) and evaluate the potential clinical benefits of early screening.
- Performance of single (blastomere) and multiple (trophoderm) cell biopsies to diagnose ADPKD.

APPENDIX A. SUPPLEMENTARY INFORMATION

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.semnephrol.2015.10.007.

REFERENCES


# DIAGNOSIS — GENETIC TESTING

**Date written:** May 2015  
**Authors:** Michel Tchan, Judy Savige, Chirag Patel, Andrew Mallett, Allison Tong, David J Tunniciffe, Gopala Rangan

Table 1. Characteristics of included studies

<table>
<thead>
<tr>
<th>Study ID</th>
<th>N</th>
<th>Study Design/ Setting</th>
<th>Participants</th>
<th>Methods</th>
<th>Results</th>
<th>Quality</th>
</tr>
</thead>
</table>
| Audrezet 2012 [4] | 700  | Mutation analysis, international, multi-centre | Patients with ADPKD from renal ultrasound. Family history of ADPKD (n=528) vs. no family history of ADPKD (n=172) | - Direct sequencing  
- Large genomic rearrangements detected by QFM-PCR.  
- Mutation pathogenic relevance was determined using bioinformatics tools | Overall detection rate of mutations was 80%  
83.8% from PKD1 and 16.2% from PKD2 (similar result to CRISP study). 37.9% frameshift, 25.3% nonsense, 17% missense mutations.  
Mutations exhibited high allelic heterogeneity in the PKD1 and PKD2 genes.  
No difference in definite pathogenic mutations in those with family history compared to those without (69.1% vs. 58.7%, P-value=0.12).  
No difference in probable pathogenic mutation in those with family history compared to those without (21.5% vs. 28.5% P-value=0.063) | Very low |

| Rossetti 2007 [3] | 239  | Mutation analysis, CRISP cohort | Patients with ADPKD aged 15-46 years old and a GFR >70 ml/min/m² at enrolment. | - Mutation analysis performed using DHPLC with amplification on PKD1 duplicated region using LR-PCR.  
- Direct sequencing on PKD1 and PKD2  
- Subset of samples screened for larger deletions | In total 81 definite mutations identified, 37 additional mutations identified.  
190 polymorphic variants in PKD1 and 8 in PKD2 | Very low |

| Rossetti 2012 [9] | 264  | Next generation sequencing | Patients with ADPKD, with unknown probands (n=230), patients with known probands of ADPKD (n=34) | - LR-PCR next generation sequencing  
- Pooling of PCR amplicons, analyzed using multiplexing bar-coded libraries vs. pooling of DNA before amplification | 234 verified variants identified  
63% confident molecular diagnosis of ADPKD  
Pooling of DNA before amplification had a sensitivity of 96% compared to pooling of PCR amplicons 81% sensitivity | Very low |

| Tan 2014 [10] | 25   | Next generation sequencing; USA, single center | Patients with ADPKD, previously undergone direct sequencing (n=25) | - Next generation sequencing—individual each patient (index test)  
- Samples had previously undergone Sanger sequencing (reference standard) | Sensitivity 99.2% (95% CI, 96.8-99.9%)  
Specificity 99.9% (95% CI, 99.7-100%) | Moderate |

| Trujillo 2014 [11] | 53  | Next generation sequencing; Spain, single center | 53 unrelated patients. Validation cohort-Patients with ADPKD (n=36) and controls (n=5), previously undergone direct sequencing. Discovery cohort—Patients with ADPKD (n=12) | - Next generation sequencing—capture and multiplex sequencing  
- Validation cohort, results compared with previous sequencing  
- Discovery cohort, results underwent bioinformatics analysis | 35 out of 36 previously known pathogenic mutations (30 PKD1, 5 PKD2) were identified  
Sensitivity and analytical specificity was 100% for both PKD1 and PKD2  
10 out of 12 patients from the discovery cohort were discovered to have a pathogenic mutation.  
11 pathogenic mutations detected in discovery cohort | Low |
Table 2. Risk of bias- Diagnostic studies- quality appraisal- QUADAS 2

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Patient selection</th>
<th>Index test</th>
<th>Reference standard</th>
<th>Flow and timing</th>
<th>Comment</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bias</td>
<td>Applicability concern</td>
<td>Bias</td>
<td>Applicability concern</td>
<td>Bias</td>
<td>Applicability concern</td>
</tr>
<tr>
<td>Tan 2014 [10]</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Risk of bias- Other studies- no quality appraisal tool available

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Comments</th>
<th>Quality</th>
</tr>
</thead>
</table>