Present & Future Role of Molecular Genetic Diagnostics in ADPKD

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Disclosure of Interests

• Otsuka Pharmaceuticals: Research grant
Mutations to *PKD1* or *PKD2* cause ADPKD


**PKD1** ~85% families

**PKD2** ~15% families
The *PKD1* region is duplicated in 16p13.1

6 *PKD1*-like pseudogenes; up to 99% identity to *PKD1*

European Consortium, Cell 1994
Sequence similarity between *PKD1* and the six pseudogenes
Sanger screening protocol for ADPKD

- 5 locus specific amplicons cover the duplicated part of PKD1
- **PKD1** exons and flanking intronic regions: 46 amplicons
- **PKD2** exons and flanking intronic regions: 17 amplicons
- Total 63 amplicons
High level of allelic heterogeneity in PKD1
Mutation Types in ADPKD: HALT PKD population

**PKD1**
- Frameshift: 30%
- Nonsense: 24%
- Splicing: 10%
- InFrame: 6%
- Large Del/Dup: 2%
- Missense: 28%

**PKD2**
- Frameshifting: 28%
- Large Del/Dup: 4%
- Splicing: 16%
- Missense: 11%
- Nonsense: 41%

No mutation identified in ~8% families

Non-definite mutations in 34% *PKD1* and 11% *PKD2*
Update to ADPKD mutation database (PKDB): Version 2.95  http://pkdb.mayo.edu

• Total 2544 variants
  – PKD1 = 2293  PKD2 = 251

• 1425 Likely Pathogenic mutations (2020 families)
  – PKD1 = 1250 (1677 families)
  – PKD2 = 175 (343 families)

• 189 Indeterminate change
  – PKD1 = 181; PKD2 = 16

• 867 Neutral Polymorphisms
  – PKD1 = 911 (94%); PKD2 = 58 (6%)
Diagnostics of ADPKD usually performed by renal imaging

- Imaging methods can usually accurately diagnose ADPKD in adults
- Imaging diagnostics less reliable in younger adults
  - Especially in families with less severe disease
    - *PKD2* and hypomorphic *PKD1* mutations
Gene-based diagnostics for ADPKD

• Genetic testing may be helpful when imaging results are equivocal and firm diagnosis required
  • Living related donors
    • Confirming negative diagnosis in young potential donor when imaging results may be unreliable
    • Clarifying the diagnosis in a potential donor when 1 or 2 cysts detected by imaging

• Individuals with a negative family history and/or an unusual disease presentation: to clarify the diagnosis
  • Early onset ADPKD
  • Mild PKD
  • Atypical radiological presentation

• Once therapies available: testing of young patients to obtain a firm diagnosis before starting treatment
Knowing the gene and the mutation are of prognostic value in ADPKD.

- PKD2 mutations
- PKD1 tronc. mutations
- PKD1 non tronc. mut.

$P < 0.0001$
Gene-based prognostics in ADPKD

- Gene and mutation data can provide information about the severity of disease
  - Truncating \textit{PKD1} mutations associated with more severe disease
  - PKD2 mutations associated with milder disease
  - Hypomorphomic \textit{PKD1} mutations associated with milder disease

- Prognostic information can help patient management
  - Planning for ESRD
  - Provide reassurance for those with predicted less severe course
  - Select patients for clinical trials
  - Select patients treatments
Resolve complex ADPKD cases

- Negative family history
  - Determine if disease is ADPKD
    - Especially with mild disease
  - De novo mutation
    - Reduced risk in sibs
    - Mosaicism – can complicate risk prediction

- Early onset cases
  - Some due to combinations of ADPKD alleles
    - Of value for avoiding further early onset cases in a family

- Marked intrafamilial variation
  - Allelic/genic combinations
  - Mosaicism
Hypomorphic *PKD1* allele in homozygosity: extreme intrafamilial phenotypic variability

Consanguineous US family of French origin homozygous or heterozygous for R3277C

Unusual, reniform kidneys with multiple small cysts in homozygotes

Rossetti et al 2009 KI
Family with \textit{PKD1} and \textit{PKD2} mutation: intrafamilial phenotypic variability

Gainullin in preparation
MLPA assay for \textit{PKD1} and \textit{PKD2}

- 3-4\% of mutations are large rearrangements

- \textit{PKD1} deletion of exons 3-9, 40\% mosaic
Screening for mosaics employing next generation sequencing

ADPKD patient with mild disease (S.Cr. 1.9 at 77y) and a negative family history was mutation negative by Sanger sequencing

NGS analysis identified the nonsense mutation *PKD1*: p.R4228X at a low level
In utero onset ADPKD

- Rarely (<1%) ADPKD presents in utero with enlarged and echogenic kidneys in a family with otherwise typical ADPKD

- These cases can be confused with ARPKD

- Increased risk of recurrence in sibs
  - Suggests simple genetic mechanism
Early-onset disease associated with co-inheritance of a truncating and hypomorphic \textit{PKD1} allele

\textit{Rossetti et al} 2009,*

\textbf{In utero onset ADPKD}

\textit{PKD1} Variants

Q2158X
R3277C
Other explanations for early onset ADPKD

- Not all EO cases due to co-inheritance of ADPKD alleles
- Co-inheritance of mutations at other loci may cause EO PKD
  - *HNF1B*
  - *PKHD1*
- Analysis of candidate panel or whole exome screen may be appropriate in unresolved EO cases
Example of combination of PKD1 and HNF1B allele causing EO PKD
Gene-based diagnostics in ADPKD is complex

- Genetic and extreme allelic heterogeneity
  - Completely screen \textit{PKD1} and \textit{PKD2} required

- Segmental duplication of \textit{PKD1}
  - Locus specific enrichment required
  - Exon capture methods unreliable

- Many variants of uncertain significance

- Many \textit{PKD1} non-truncating changes hypomorphic
  - Identification of hypomorphic alleles difficult

- Genetic test is expensive and not always informative

- Reports often uninformative and difficult to understand
  - Clinical testing only available through one vendor in US:
    - Athena Diagnostics - \$5000 with MLPA testing
  - Recent Supreme Court ruling may open US market
Next-generation sequencing allows rapid analysis of multiple patient samples

• **PKD1** and **PKD2** amplified as 14 long-range products
  • exon capture unreliable for **PKD1** because of genomic duplication

• Potential for higher throughput and reduced cost

• Mutation detection rate likely to be comparable

• Introns, UTRs and promoters could also be screened
Molecular Diagnosis of Autosomal Dominant Polycystic Kidney Disease Using Next-Generation Sequencing

Adrian Y. Tan,* Alber Michael, * Genyan Liu,* Olivier Elemento, † Jon Blumenfeld, †§ Stephanie Donahue, § Tom Parker, § Daniel Levine, § and Hanna Rennert,*

Table 4  NGS Analytic Sensitivity and Specificity (Variants Detection)

<table>
<thead>
<tr>
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<th>Sanger sequencing</th>
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<tr>
<td></td>
<td>Variant alleles</td>
<td>Reference alleles</td>
<td>Total</td>
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<tr>
<td></td>
<td>(positive)</td>
<td>(negative)</td>
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<tr>
<td>NGS</td>
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<td>Variant alleles (positive)</td>
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<td>Reference alleles (negative)</td>
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<td>Total</td>
<td>250</td>
<td>1825</td>
<td>2075</td>
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Table 6  Comparison of Reagents, Sequencing Costs, and Time of Labor for Sanger Sequencing and NGS

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<th>Method</th>
<th>Purpose</th>
<th>Quantity</th>
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<tr>
<td></td>
<td></td>
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<td>Per sample</td>
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<td>Per subject</td>
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<td>Sanger sequencing (N = 25)</td>
<td>LR-PCR (PKD1)</td>
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<td>Standard PCR (PKD2)</td>
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<td>Purification</td>
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<td>NGS (N = 25)</td>
<td>LR-PCR (PKD1 and PKD2)</td>
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<td>Data analysis</td>
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<tr>
<td>Total</td>
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<td>2050.00</td>
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Mutation-based diagnostics in ADPKD is likely to be more widely employed

- Mutation identified in 90% cases
  - Definite (truncating) mutations in ~65% families
- Bioinformatic scoring of non-definite mutations increasingly reliable
  - Recurrent mutations ~50% in recent studies
  - Mutation database of value
    - Identify pathogenic mutations
    - Highlight hypomorphic changes
- Of diagnostic and prognostic value
- Mutation type may in the future influence treatment options
  - Similar to cystic fibrosis
- Cost of test needs to decrease
- Reliability and interpretation of results needs to improve