Cyst Number but Not the Rate of Cystic Growth Is Associated with the Mutated Gene in Autosomal Dominant Polycystic Kidney Disease

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Data from serial renal magnetic resonance imaging of the Consortium of Radiologic Imaging Study of PKD (CRISP) autosomal dominant polycystic kidney disease (PKD) population showed that cystic expansion occurs at a consistent rate per individual, although it is heterogeneous in the population, and that larger kidneys are associated with more rapid disease progression. The significance of gene type to disease progression is analyzed in this study of the CRISP cohort. Gene type was determined in 183 families (219 cases); 156 (85.2%) had PKD1, and 27 (14.8%) had PKD2. PKD1 kidneys were significantly larger, but the rate of cystic growth (PKD1 5.68%/yr; PKD2 4.82%/yr) was not different (P = 0.24). Cyst number increased with age, and more cysts were detected in PKD1 kidneys (P < 0.0001). PKD1 is more severe because more cysts develop earlier, not because they grow faster, implicating the disease gene in cyst initiation but not expansion. These insights will inform the development of targeted therapies in autosomal dominant PKD.


Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive cyst development and expansion, resulting in ESRD in the majority of patients (1,2). ADPKD is genetically heterogeneous with two loci identified—PKD1 (16p13.3) and PKD2 (4q21)—that encode the proteins polycystin-1 and polycystin-2 (3–5). The majority of patients (approximately 85%) have PKD1, with PKD2 accounting for most of the remainder (6–8). Genetic modifying factors, as well as the environment, also significantly influence the course of this disease (9,10). On the basis of detected somatic mutations in isolated cystic linings and cell lines that are derived from single cysts, plus animal models, it has been proposed that cyst initiation is a two-hit process (11–15). However, dosage changes of a polycystin molecule also may result in cyst development (16–20), and heterogeneity of a developing cyst questions whether a second hit is always necessary as an initiating event (21).

PKD2 is consistently a milder disease as evidenced in age at ESRD (PKD1 54.3 yr; PKD2 74 yr) and age at diagnosis of the disease and of hypertension (7,22,23). The Consortium of Radiologic Imaging Study of PKD (CRISP) was established to determine from a prospective, longitudinal study whether radiologic measures of kidney and cyst volumes by magnetic resonance imaging (MRI) could be used as an early means to monitor disease progression (24). This study showed that kidney and cyst volumes increase in most patients and that larger kidneys are associated with a decline in renal function (25). Previously, no significant difference was found between PKD1 and PKD2 kidneys by ultrasound analysis (7,26), but preliminary data from the CRISP study (before the genotyping was complete) showed that PKD1 kidneys are significantly larger than PKD2, consistent with correlations between renal size and function (25). Here, with completed genotyping data, PKD1 and PKD2 kidneys are compared in more detail, and insights are provided about the process of cystogenesis.
Materials and Methods

Radiologic and Statistical Analyses

Detailed descriptions of the structure of this study, the baseline characteristics of the cohort, and details of evaluations during the course of the study have been published previously (24–27). Patients who had ADPKD (n = 241), were 15 to 46 yr of age, and had a GFR of >70 ml/min at enrollment were evaluated at baseline and annually over 3 yr for renal function and MRI of the kidney with coronal T1- and T2-weighted images to calculate renal and cyst volumes. Volumes were measured on a 3-mm slice-by-slice basis by two analysts who were blinded to genotype, as described previously in detail (24). The annual percentage change of total kidney and cyst volume was determined by regressing log-transformed (on a base-10 scale) against time (baseline to year 3) for individuals using a mixed linear model. To count the number of cysts, we chose a middle section of the left kidney on coronal T2-weighted images, and any cyst with a diameter of ≥4 mm was recorded by a single analyst who was blinded to genotype.

The percentage change of corrected iothalamate clearance was calculated by dividing the slope estimate by the intercept value to measure GFR. Logistic regression was used to evaluate hypertension in PKD1 and PKD2 groups after adjustment for gender and age. Statistical methods used in this study included mixed-model ANOVA (and t test). Cross-tabulation comparisons were examined using χ2 methods.

Genotyping Analysis

Details of the genetic study will be described elsewhere. Briefly, samples for genotyping were available from 239 patients from 202 different families. The PKD1 and PKD2 genes were screened in each family by denaturing HPLC (28), and mutation-negative cases (plus controls) were analyzed using a commercial diagnostic test (Athena Diagnostics, Worcester, MA) that uses direct sequencing. Larger deletion mutations also were sought by field inversion gel electrophoresis (29). The overall detection rate was 90.1%. Linkage analysis with markers flanking PKD1 and PKD2 also was used to identify gene type in three large families in whom no mutation was detected.

Results

Mutation analysis of the CRISP cohort identified likely pathogenic changes in 180 pedigrees (211 patients) with linkage identifying the gene in three more families (eight patients). Twenty-seven (14.8%) pedigrees (34 patients) had PKD2, and 156 (85.2%) pedigrees (185 patients) had PKD1, a distribution similar to that previously found in clinical ADPKD populations (6,7). Despite that the patients with PKD2 were significantly older, they were less likely to be hypertensive and had smaller kidney and cyst volumes at baseline (Table 1, Figure 1, A and B). The age- and gender-adjusted PKD2 kidney and cyst volumes were, respectively, 59.8 and 43.2% of their PKD1 counterparts. Although there was no difference in GFR or renal blood flow (another early marker of kidney function [30]) between PKD1 and PKD2 at baseline, there was significantly more urinary albumin in PKD1 cases (Table 1). Kidney and cyst volumes consistently increased in both the PKD1 and PKD2 populations, and the absolute rate of change was greater in PKD1 (25) (Figure 1, A through D, Table 1). However, this was due to the larger baseline sizes of the kidneys; the rates of growth for kidney and cyst volume were not significantly different (Table 1, Figure 1, A through D). This indicates that gene type does not strongly influence the size of ADPKD kidneys by modulating the relative rate of cyst growth. Gender, however, was associated with both the absolute and relative rates of kidney and cystic expansion in the ADPKD population, with male patients showing more rapid expansion (Table 2).

Analysis of cyst number at baseline in all patients showed that PKD2 kidneys have significantly fewer cysts (55.9% of those found in PKD1 kidneys) and that there is a correlation between cyst number and kidney volume (Figure 1, E and F, Table 1; see the Materials and Methods section for details). In both PKD1 and PKD2, the number of cysts was correlated with the age of the patient, illustrating that new cysts develop during the course of the disease. Although the slopes of the regression lines that depict the relationship between age and cyst number are not significantly different between the PKD1 and PKD2 populations (P = 0.77; Figure 1E), the intercept to the y axis is significantly lower for PKD2 (approximately 14 cysts per MRI section; P < 0.0001), suggesting more aggressive early onset of cystogenesis in PKD1. Representative MRI images showing examples of younger and older PKD1 and PKD2 kidneys illustrate the differences in terms of cyst number, as well as total cystic volume (Figure 2), although there is considerable heterogeneity within each of the genic populations (Figure 1, E and F). Overall, these data indicate that PKD2 kidneys are smaller because they develop fewer cysts, especially at the early stages of the disease.

Discussion

Hypertension and urinary albumin excretion were significantly more common in PKD1 than PKD2, consistent with these variables’ being associated with more severe disease (24,25). However, the major new conclusion from this study is that the genic effect is at the level of cyst initiation; the rate of cystic enlargement is not modulated by the disease gene. Although it is logical that the disease mutation is involved in cyst initiation, similar rates of cystic growth in the two disorders has not been shown previously. Therefore, two distinct phases to cystogenesis, a disease gene–related initiation phase and a gene-independent cyst enlargement phase, have been defined.

Gender was associated with the rate of cyst expansion in the total cohort. Previously, male individuals have been associated with more severe disease in ADPKD (2), and although this has been demonstrated in PKD2 (31), recent data on PKD1 have not shown a significant difference (22,32) in age at ESRD. Our data indicate that gender may be important, however, to the rate of cystic expansion, suggesting a hormonal influence on the process. The faster expansion of cyst volume in male individuals is consistent with the stimulating effect of testosterone on cAMP accumulation and chloride and fluid secretion by MDCK cells (33). A hormonal effect was identified previously in polycystic liver disease, in which more severe disease in women is thought to be promoted by estrogen exposure (34,35).

Our data lead us to suggest that new cysts develop during the life of the patient, although the expansion of microscopic cysts initiated in utero (36) to a level where they are recorded (≥4 mm), may be significant; and differential rates of growth of PKD1 and PKD2 microcysts cannot be ruled out. That cysts continue to develop in childhood and adulthood also is indi-
cated because, although the rate of cystic expansion is similar in both kidneys in an individual (25), there is considerable heterogeneity in cyst size (Figure 2). These concepts are presently being tested in conditional mouse models of ADPKD (37) and by further observations of the CRISP cohort. Because PKD1 kidneys have more cysts even at young ages (Figure 1, E and F), the rate of cyst initiation at early ages, including in the fetus, may be important.

The reason that fewer cysts develop in PKD2 is not known, but it seems to fit neatly with the concept that cystogenesis is a two-hit process that requires a somatic mutation for cyst initiation (11). Several factors suggest that the PKD1 gene may sustain a higher level of somatic insults than PKD2. Most notable among these is the larger size of the coding region (approximately 12.9 kb compared with approximately 3 kb) and the GC richness of the DNA, resulting in a higher level of CpG dinucleotides that are known warm spots for mutations (38). In addition, special factors, such as a polypurine tract in IVS21 and six pseudogenes that match much of the 5’ two thirds of PKD1, may increase the somatic mutation level at PKD1 (29,39,40). A significant level of de novo germline mutations at PKD1 emphasize that new mutations occur at a significant level at this locus (38). It is possible, however, that there are other reasons that a PKD1 germline mutation might be more likely to result in cyst development. For example, polycystin-1 may be more important during renal development than polycystin-2, or some PKD1 mutations may generate stable proteins that can act as dominant negatives and thus have an enhanced detrimental effect.

A comparison of patients within the PKD1 or PKD2 populations show that there is considerable variability in the rate at which cysts grow (Figure 1, C and D), although similar individual rates are found for the right and left kidneys (25). This

| Table 1. Comparison of PKD1 versus PKD2 imaging/clinical parametersa |
|--------------------------|--------------------------|--------------------------|
| Baseline values          | PKD1  (n = 185)          | PKD2  (n = 34)          | p0     |
| Hypertension             | 120 (65%)                | 17 (50%)                | 0.0998 |
| Mean age (yr)            | 31.77                    | 35.42                   | 0.0281 |
| Mean kidney volume (ml)  | 1010.90                  | 688.11                  | <0.0001|
| Mean cyst volume (ml)    | 384.03                   | 199.26                  | 0.0011 |
| Mean GFR (ml/min per 1.73 m2) | 96.92                 | 96.67                   | 0.96   |
| Mean 24-h urinary albumin (g/L) | 27.94               | 16.59                   | 0.0108 |
| Mean number of cystsc   | 30.79                    | 18.76                   | <0.0001|
| Total RBFd (ml/min per 1.73 m2) | 731.1                 | 731.02                  | 0.9264 |
| PKD1 n = 85; PKD2 n = 15 | 734.75                  | 752.34                  | 0.7681 |

<table>
<thead>
<tr>
<th>Changes per yeare (n = 179)</th>
<th>(n = 33)</th>
<th>p0</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>-1.11</td>
<td>-0.89</td>
</tr>
<tr>
<td>Rate of kidney volume change (%)</td>
<td>5.55</td>
<td>4.69</td>
</tr>
<tr>
<td>Cyst volume change (ml)</td>
<td>70.69</td>
<td>28.72</td>
</tr>
<tr>
<td>Rate of cyst volume change (%)</td>
<td>12.21</td>
<td>11.40</td>
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</tbody>
</table>

aValues adjusted for age, gender, and the age and gender interaction (except for mean age, adjusted for gender only) are shown in italics. PKD, polycystic kidney disease; RBF, renal blood flow.
bSignificant P values are in boldface.
cMeasured from representative magnetic resonance imaging (MRI) section (see Materials and Methods for details).
dRBF was available only on a subset of cases.
eMean calculated over 3 yr of follow-up.
rate seems to be independent of the disease gene and reflects genetic modifying effects, as well as environmental influences and gender. Similarly, considerable variation in cyst number is seen within the PKD1 and PKD2 populations (Figure 1, E and F), probably influenced by allelic effects, genetic modifiers, the environment, and stochastic factors on the rate of cyst initiation. These findings have implications for identifying quantitative trait loci that modulate disease severity and the development of effective therapeutics.

**Conclusion**

We have defined cystogenesis as a two-phase process: Cyst initiation, associated with the disease mutation, and cyst expansion, which is disease gene independent. Both phases vary between individuals. Therefore, quantitative trait loci or potential therapies may have an influence on the rate of cyst formation by preventing somatic mutations or by regulating the growth of cysts. Assuming that the downstream changes that are associated with cystogenesis as a result of disruption of the
polycystin complex are similar in PKD1 and PKD2, it is likely that factors that target cystic growth may be equally effective in both disorders. Most therapies that presently are under consideration, such as the clinical evaluation of vasopressin receptor antagonists (41), fall into this second group.

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References

Table 2. Comparison of gender with baseline values and measures of disease progression in the total ADPKD population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male (n = 88)</th>
<th>Female (n = 131)</th>
<th>P*b</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean age (yr)</td>
<td>33.43</td>
<td>33.31</td>
<td>0.8224</td>
</tr>
<tr>
<td>kidney volume (ml)</td>
<td>861.52</td>
<td>751.00</td>
<td>0.0441</td>
</tr>
<tr>
<td>cyst volume (ml)</td>
<td>266.90</td>
<td>253.67</td>
<td>0.7028</td>
</tr>
<tr>
<td>cyst numberc</td>
<td>25.86</td>
<td>22.59</td>
<td>0.0847</td>
</tr>
<tr>
<td>kidney volume change (ml)</td>
<td>66.28</td>
<td>40.41</td>
<td>0.0062</td>
</tr>
<tr>
<td>rate of kidney volume change (%)</td>
<td>5.88</td>
<td>4.59</td>
<td>0.0163</td>
</tr>
<tr>
<td>cyst volume change (ml)</td>
<td>63.01</td>
<td>36.04</td>
<td>0.0039</td>
</tr>
<tr>
<td>rate of cyst volume change (%)</td>
<td>13.83</td>
<td>10.66</td>
<td>0.0029</td>
</tr>
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</table>

*Values are adjusted for age and genotype. ADPKD, autosomal dominant PKD.
*Significant P values are in boldface.
*Measured from representative MRI sections (see Materials and Methods for details).
*Mean calculated over 3 yr of follow-up.

Figure 2. Coronal T2-weighted, single-shot fast spin echo magnetic resonance images from patients with PKD1 (a and b) and PKD2 (c and d) at 18 yr (a and c) and 41 yr (b and d). Renal cysts in PKD1 are more numerous, diffusely distributed, and heterogeneous in size than those in PKD2.


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