Linkage, Clinical Features, and Prognosis of Autosomal Dominant Polycystic Kidney Disease Types 1 and 2

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ABSTRACT

Linkage analysis was performed on 49 Catalan families with autosomal dominant polycystic kidney disease obtained via the Nephrology Department and related nephrology centers. A total of 336 subjects, 267 at risk for the disease, were investigated using three microsatellites linked to polycystic kidney disease Type 1 (PKD1) and three microsatellites linked to PKD2. All of the subjects underwent physical and sonographic examination. The results demonstrate the maximum likelihood for the proportion of families linked to PKD1. All of the remaining families were found to be linked to PKD2. Analysis of clinical data in the PKD1 group (N = 146) versus the PKD2 group (N = 20) showed a milder form of the disease in the latter, with a lower age at diagnosis (27.4 versus 41.4 yr, P = 0.0002), later age of onset of ESRD (53.4 versus 72.7 yr, P = 0.0001), and lower prevalence of hypertension at younger ages. Sonographic findings did not differ significantly between groups. Although the majority of families. No signs of imprinting were found in this study, and the only gender effect was an earlier age of onset of ESRD in men than in women (49.5 versus 53.1 yr in PKD1, P < 0.01 and 70.57 versus 73.6 yr in PKD2, P = 0.1). Molecular analysis of autosomal-

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lower age of onset of ESRD in PKD1 than in PKD2 (23-30). However, sonographic and clinical data have scarcely been reported for PKD2.

We present here the clinical and molecular analysis of ADPKD families from Catalonia, in the northeast region of Spain. We studied 49 informative families with ADPKD by using the most informative and recently described microsatellites, with the following goals: (1) to establish the genetic heterogeneity of ADPKD in our population, (2) to assess the sensitivity and specificity of ultrasonography and genetic linkage studies for presymptomatic diagnosis in PKD1 and PKD2, and (3) to determine the differences between PKD1 and PKD2, based on clinical and sonographic features.

MATERIALS AND METHODS

Families and Clinical Evaluation

Families were obtained via the Nephrology Department from the Hospital Clinic of Barcelona and related nephrology centers. Each family included at least three affected members or two affected and two unaffected members. A total of 336 individuals belonging to 49 unrelated ADPKD families were included in this study. Informed consent was obtained before investigation. Forty-two patients had severe renal impairment and were on renal replacement therapy. All of the members of the families, including spouses, were physically and ultrasonographically examined at our hospital.

Diagnostic criteria included the presence of at least two cysts in one kidney or one cyst in each kidney in an at-risk person under 30 yr of age, the presence of at least two cysts in each kidney in an at-risk person between 30 and 59 yr of age, and at least four cysts in each kidney for those persons at risk aged 60 yr and older (6). Ultrasound examinations were carried out by a single consultant sonographer in all cases using a Toshiba 3.5- or 5-MHz linear array or sector probe (Paris, France).

The physical examination included measurement of arterial blood pressure and serum creatinine level. A patient was considered to be hypertensive if the systolic or diastolic blood pressure exceeded the 95th percentile for his or her age and sex (31) or if he or she had been previously diagnosed and treated for this condition. Serum creatinine level was measured with an automatic analyzer. Creatinine clearance rate was calculated according to the formula of Cockcroft and Gault. Renal impairment was indicated by a creatinine clearance rate of less than 70 mL/min or the presence of ESRD. Any history of renal calculi (passage of a stone with recovery of the calculus or evidence of calculi within the collecting system reported by the radiologist), urinary tract infections (lower urinary tract symptoms together with a positive diagnosis of cystitis or temperature together with the diagnosis of pyelonephritis or cyst infection—positive blood or urine cultures were not required for the diagnosis), and ruptured cerebral aneurysms assessed with an arteriography, was ascertained by the nephrologist during the interview of each family member. Pedigrees were drawn on the basis of the same interview. Age of diagnosis of ADPKD or hypertension was considered to be the age at which a subject was first informed that he or she had ADPKD or high blood pressure. The age of onset of ESRD was taken to be the age at which long-term replacement therapy for renal function became necessary or, in persons who died before such treatment became available, the age of death as a result of uremia (23,32). Anticipation, in a family, was defined as at least a 10-yr-earlier onset of ESRD in the offspring of any parent-offspring pair (32). This study was approved by the ethics and clinical trials committee of the Hospital Clinic.

DNA Analysis

Twenty milliliters of EDTA-anticoagulated peripheral blood was removed from each family member. DNA was extracted according to the salting-out method (33). ADPKD families were typed using three (CA), microsatellites for PKD1 (AC2.5-D16S291, KG8-PKD1, and CW2-D16S663) and three (CA), microsatellites for PKD2 (D4S423, D4S1534 and D4S1542). KG8 is an intragenic marker, at the 3' end of the PKD1 gene, whereas the other two Chromosome 16 microsatellites are proximal to PKD1 (Figure 1). Polymerase chain reaction amplification was performed as described in previous reports.

![Figure 1. Schematic representation of the chromosomal regions containing the PKD1 and PKD2 loci. The distances between the genetic markers are indicated approximately for the PKD1 locus in megabases (Mb) and for the PKD2 in centimorgans (cM). The exact location of the PKD2 gene, with respect to the three markers, is unknown.](image-url)
(34–37). The methods used to develop the ADPKD microsatellite analysis have been the silver staining (38) and the enhanced chemiluminescence (39) techniques (Figure 2).

**Linkage Analysis and Statistical Methods**

We performed linkage analysis using the lod score method (40) to estimate the recombination fractions (θ) between the disease locus PKD1 and markers AC2.5, KG8, and CW2, in a two-point analysis. Also, we performed θ estimations for PKD2 and markers D4S423, D4S1534, and D4S1542. To calculate the different θ values and their respective lod scores (Z(θ)), we used the computer programs MLINK and ILINK from LINKAGE software for PC DOS V5.2 (Lathrop). In the calculations, we took into account gene frequencies of 0.001 for PKD1 and 0.0001 for PKD2. The allele frequencies for DNA markers were taken from published data for the Spanish population (35). Alternatively, given the negligible genetic distance between the above-mentioned markers (X = θ = 0), we constructed haplotypes from them and down-coded as a new refined marker to increase the marker informativeness. In this case, we assumed equal allele frequencies. The Z(θ) values were calculated both with and without penetrance value incorporation (liability classes LC). Three LC were used to account for age-dependent penetrance, according to the cumulative frequency of age of onset curve obtained from published data (23,24,28,41,42): LC1 = 0.64 (<20 yr); LC2 = 0.92 (20 to 30 yr); LC3 = 1 (>30 yr). No differences in recombination fractions between sexes and the absence of genetic interference and spontaneous mutations were assumed in the calculations.

We estimated the proportion of linked families, α, and the θ values between PKD1 and our 16p markers, including the downcoden one, according to the maximum likelihood estimate (MLE) method of homogeneity test, using the HOMOG program PC DOS V 3.3 (40). The “final lod scores” included in the input table were obtained by subtracting the Log 10 likelihood value at θ = 0.5 from the other different Log 10 likelihood values at θ of 0, 0.001, 0.05, 0.1, 0.2, 0.3, and 0.4. Thus we obtained the MLE among α and θ from our family data. The results were displayed with a three-unit support interval (ln likelihood ratio = 3, corresponding to 1.3 units of lod score).

The t test for independent populations was used to compare continuous variables. Two-tailed chi-squared tests were used to compare qualitative variables. In some cases, when the number of individuals in a group was low, the Yates correction was performed, and the results were confirmed with Fisher’s Exact Test (2 × 2 tables). To compare the renal size related to the age, between PKD1 and PKD2, a covariance analysis, taking age as the concomitant variable, was performed. The survival time to onset of ESRD was calculated through the method of product limit (Kaplan Meier) (43). To compare the survival time between PKD1 and PKD2 and between men and women, several tests were performed:

![Image of pedigrees and microsatellite analysis](image)
Mantel-Cox, Tarone-Ware, Breslow, and Peto-Prentice. The computer programs used to perform the statistical analysis were STATGRAPHICS (Statistical Graphics Corp., Princeton, NJ) for chi-squared, Fisher's Exact Test, and covariance analysis and 1L program for the BMDP statistics package (BMDP Statistical Software Inc., Los Angeles, CA) for survival analysis.

RESULTS

Linkage Analysis

Eight families were initially discarded because of lack of informativity. A total of 336 subjects, 267 of whom were at risk, belonging to 49 families, were characterized for the six DNA microsatellites. The two-point analysis between 16p markers AC2.5, KG8, and CW2 and the downcoded haplotype with respect to PKD1 gives the corresponding lod scores of 16.32, 14.78, 7.56, and 28.08 at the respective \( \theta \) values of 0.07, 0.06, 0.07, and 0.05, not taking into account LC. and 16.24, 14.48, 7.04, and 27.62 at \( \theta \) values of 0.059, 0.04, 0.07, and 0.037, taking into account LC. The results of the pairwise analyses between ADPKD and each marker locus gave strong evidence in favor of linkage to PKD1 in 44 families and against linkage in five families. The analysis of our pedigrees with the 16p-downcoded haplotype with the homogeneity test gave a MLE at \( \alpha = 0.85 \) and \( \theta = 0 \), not taking into account LC, and \( \alpha = 0.90 \) and \( \theta = 0 \), not taking into account LC, showing linkage and heterogeneity (alternative hypothesis \( H2 \)) with a very high degree of significance \( (P < 0.001 \) in both cases). The three-unit support intervals were \( \alpha \) and \( \theta \); 0.7, 0, and 0.95, 0.01 when not taking into account LC, and 0.8, 0.000, and 0.995, 0.001 when taking into account LC. We had very similar results when we used other 16p markers. These results are in agreement with most previous reports (23,24,28,42). The test produces an output result that shows the conditional probability of being a linked family type according to a Bayesian calculation (with prior probability to be linked at \( \theta = 0 \) of \( \alpha = 0.85 \)).

We performed a two-point analysis between PKD2 and 4q markers in families that had a conditional probability of linked type of \( P = 0 \) and additional pairwise lod score of \( Z (0) < -2 \) from the above-mentioned 16p markers. These families without linkage to 16p markers gave a lod score of 5.68 at \( \theta = 0 \), taking into account penetrance, and 5.13 at \( \theta = 0.032 \), not taking into account penetrance, to the downcoded haplotype 4q marker (constructed from D4S423, D4S1534, and D4S1542), indicating that the disease locus in these pedigrees is located very close to the above-mentioned region on Chromosome 4q.

After the linkage analysis, four groups of subjects were established: PKD1 \( (N = 146; 64 \) men, 82 women; mean age, 35.41; SD, 17.8 yr), nonaffected PKD1 family members \( (N = 128; 62 \) men, 66 women; mean age, 38; SD, 17.6 yr), PKD2 \( (N = 20; \) eight men, 12 women; mean age, 44.5; SD, 22.7 yr) and nonaffected PKD2 family members \( (N = 22; \) ten men, 12 women; mean age, 41; SD, 18.9 yr). No significant differences related to age or sex were found among any of the studied groups.

The allele frequencies in the Catalan population were similar to those found in other European and Spanish populations (35,36). None of the microsatellites analyzed for PKD1 displays a statistically significant difference in allele distribution between PKD1 and control chromosomes. One recombination event in PKD2 microsatellites, between D4S1542 and D4S423, was detected. Another recombinant was found for PKD1 between CW2 and AC2.5.

Age and Cause of Diagnosis

One hundred forty-six PKD1 and 20 PKD2 patients were asked about the age and reason of diagnosis of the disease. The age of diagnosis differed significantly between both groups, the PKD1 patients being diagnosed earlier than the PKD2 patients: 27.4 ± 13.4 yr \textit{versus} 41.4 ± 16.9 yr \( (P = 0.0002) \). The principal cause of diagnosis in both groups was screening as a result of family history of ADPKD. The rest of causes were similar in both groups (Table 1), with a slightly lower prevalence of symptomatic causes in PKD2. In this study, we diagnosed 36 PKD1 patients and six PKD2 patients, who previously did not know that they were affected.

Hypertension

Hypertension was significantly more prevalent among ADPKD-affected subjects than among nonaffected family members in subjects over 20 yr of age (Table 2). Although PKD1 patients seem to have a major prevalence of hypertension, especially at younger ages, the statistical tests were not significant because of the low number of individuals in the PKD2 group. The age of diagnosis of hypertension differed significantly in both groups. PKD1-hypertensive subjects were diagnosed earlier than PKD2-hypertensive subjects: 34.8 ± 10.9 yr \textit{versus} 49.7 ± 12.1 yr \( (P = 0.001) \).

Table 1

Factors leading to the diagnosis of PKD1 and PKD2 in affected subjects

<table>
<thead>
<tr>
<th>Factor</th>
<th>PKD1 (%)</th>
<th>PKD2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family History</td>
<td>38 (26)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (10.3)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Back Pain</td>
<td>12 (8.2)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Urinary Tract Infections</td>
<td>10 (6.8)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>High Creatinine Levels</td>
<td>9 (6.2)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Renal Calculi</td>
<td>6 (4.1)</td>
<td>0</td>
</tr>
<tr>
<td>Routine Sonography</td>
<td>2 (1.4)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Abdominal Mass</td>
<td>2 (1.3)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>4 (2.7)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Molecular Analysis</td>
<td>36 (24.6)</td>
<td>6 (30)</td>
</tr>
</tbody>
</table>

*(PKD1 and PKD2, polycystic kidney disease Types 1 and 2.*
Table 2
Prevalence of hypertension, renal calculi, and urinary tract infections in PKD1 and PKD2 subjects

<table>
<thead>
<tr>
<th>Condition</th>
<th>Age of Subject</th>
<th>PKD1 Nonaffected Family Members</th>
<th>PKD1</th>
<th>PKD2</th>
<th>PKD2 Nonaffected Family Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBP (%)</td>
<td>&lt;20 yr</td>
<td>0/25 (0)</td>
<td>4/36 (11.1)</td>
<td>0/4 (0)</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td>HBP (%)</td>
<td>21 to 40 yr</td>
<td>1/45 (2.2)</td>
<td>16/49 (32.6)</td>
<td>1/6 (16.6)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>HBP (%)</td>
<td>&gt;40 yr</td>
<td>18/58 (35.3)</td>
<td>48/61 (78.7)</td>
<td>7/10 (70)</td>
<td>4/9 (44.4)</td>
</tr>
<tr>
<td>Renal Calculi (%)</td>
<td>13/128 (10.1)</td>
<td>26/146 (17.8)</td>
<td>3/20 (15)</td>
<td>(P = 0.001)</td>
<td>2/22 (9)</td>
</tr>
<tr>
<td>Urinary Tract Infections (%)</td>
<td>22/128 (17.1)</td>
<td>51/146 (34.9)</td>
<td>6/20 (30)</td>
<td>(P = 0.06)</td>
<td>4/22 (18.2)</td>
</tr>
</tbody>
</table>

aHBP, hypertension.
bThe χ² test has been performed with the Yates correction, because of the low theoretical frequency of some samples; the significance of the test has been checked with the Fisher’s Exact Test.

Renal Calculi, Urinary Tract Infections, and Cerebral Aneurysms

Renal calculi were more prevalent in ADPKD patients than in nonaffected family members, although the differences were not significant, whereas urinary tract infections are significantly more prevalent in PKD1 patients than in nonaffected family members. This is basically a result of the inclusion of cyst infections in ADPKD subjects. Neither renal calculi nor urinary tract infections seem to be more frequent in any type of ADPKD (Table 2), but our data cannot answer this question definitively, because the number of PKD2 patients is not large enough.

We found five PKD1 patients (four of them belonging to two different families) diagnosed with ICA and no PKD2 patient with this abnormality.

End-Stage Renal Disease

Thirty-eight (26%) of the PKD1-affected subjects had ESRD, with the earliest age of onset at 32 yr. Four (20%) of the PKD2-affected subjects had ESRD, with the earliest age of onset of ESRD at 53 yr. Figure 3 shows the cumulative survival to the time of onset of ESRD of affected members of PKD1 and PKD2 families. The mean survival to the onset of ESRD was shorter among PKD1 patients than among PKD2: 53.5 (SE, 0.97) versus 73.7 (SE, 2.6) yr (P < 0.001). The median survival was 52 yr (SE, 1.0; 95% confidence interval [CI], 50 to 54) for PKD1 and 71 yr (SE, 0.9; 95% CI, 69 to 73) for PKD2. Twenty-five percent of PKD1 patients had not reached ESRD by the age of 59, whereas the same percentage of PKD2 patients were free of ESRD by the age of 74 (P < 0.001). Among PKD1 subjects, men had a lower survival rate than women: 49.6 (SD, 9.6) versus 53.1 (SD, 9.1) yr (P < 0.01), and the same was true for PKD2 subjects: 70.6 (SD, 5.3) for men and 73.6 (SD, 13.5) yr for women (P = 0.1).

We studied the presence of anticipation among 35 PKD1 and three PKD2 families with more than one generation with reliable data of onset of ESRD. We found eight PKD1 families and two PKD2 families that accomplished the criteria for anticipation. When we analyzed each parent-offspring pair, we found 27 of 51 pairs showing anticipation in the PKD1 families and three out of seven pairs showing anticipation in PKD2 families (Figure 4). We found no gender effect in the parent-offspring pairs with anticipation: in 14 of the 27 PKD1 pairs and in two of the three PKD2 pairs, the mother was the carrier of the disease (Figure 4).

Ultrasoundographic Diagnosis

Little discordance was observed between ultrasound findings and haplotype segregation. Ultrasonographic examination of 121 individuals younger than 30 yr with 50% risk of ADPKD disclosed the high sensitivity and specificity of this technique (Table 3). Among 108 at-risk members of PKD1 families who were under 30 yr of age, 64 had ADPKD. Among 13 members of PKD2 families at risk who were under 30 yr of age, six had ADPKD. In 267 individuals with 50% risk of ADPKD, ultrasonographic diagnoses were compared with genotypes inferred from linkage studies. Discordance between negative ultrasonographic scan findings and genotypes was age-dependent. Of 64 persons under 30 yr of age who inherited the PKD1 mutation, two did not have renal cysts (2 and 5 yr of age), and two had an equivocal ultrasonographic result (3 and 26 yr of age); of 82 patients of over 30 yr of age, all had cysts on ultrasonographic examination. It is worthy noting that the 26-yr-old girl who had no cysts had an affected sister and two affected cousins in their 20s with very few cysts. Of six persons under 30 yr of age who inherited the PKD2 mutation, two did not have renal cysts (5 and 11 yr of age), and, of 14 patients of older 30 yr of age, all had cysts on ultrasonographic examination. Among the nonaffected members of the families, we found a prevalence of simple renal cysts of 13.7%, all of the cysts being
found in subjects older than 40 yr. The prevalence of hepatic cysts in the nonaffected population was much lower (4%). The kidney size (mean renal bipolar diameter) for the different groups of age was compared between PKD1 and PKD2 patients, with no significant differences. In both groups, the kidney size was significantly influenced by the age. The prevalence of hepatic cysts was similar in PKD1 and PKD2 patients, and, in both groups, it was directly related to the age (Table 3). Only 50% (32 of 64) of male subjects had hepatic cysts in PKD1 compared with 61% (50 of 82) of female subjects ($P = 0.18$). No pancreatic cysts were detected in PKD2 patients, whereas the prevalence of this type of cyst in PKD1 patients was 4.2%.

**DISCUSSION**

ADPKD is a systemic disease with variable clinical features among the different affected individuals. At this time, we do not know whether this phenotypic heterogeneity is caused by genetic differences or whether it is a result of the interaction of environmental agents. If the clinical heterogeneity is caused by the effects of different mutations at the same locus, we would expect that there should be minimal variation of clinical features within a family; otherwise, if those phenotypic differences are the result of environmental agents interacting with the disease locus, the pattern of the disease should vary significantly within and among families. Furthermore, other genetic factors might also contribute to the clinical variations observed among members of the same family. The results presented in this study provide evidence for clinical and genetic heterogeneity in ADPKD.

Five of the 49 families studied were proven to be linked to the PKD2 locus, resulting in a prevalence of PKD2 of 15%. This prevalence is similar to that reported by Peters et al. (17) but differs significantly from that reported by other authors (5,16,22,23). The difference in the two forms of ADPKD is probably a result of population genetics and/or ascertainment bias. This bias could be expected from the easier access to PKD1 families as a result of their higher morbidity and their consequently higher presence in hospitals, and thus the prevalence of this form of
ADPKD may be higher than the 15% observed. It is noteworthy that all studied families were linked either to PKD1 or PKD2; therefore, the prevalence of families not linked to any of these loci (20,21) is expected to be extremely low. Evidence for linkage disequilibrium has been found for two markers: VK5 (D16S94), in Scottish ADPKD families (44), and Blu24 (D16S662), in Spanish ADPKD families (35). Snarey et al. (36) also pointed out a significant association between some microsatellites and the disease, but did not find a founder haplotype. In our series, we have not studied these markers, but we found no evidence of specific association between an allele of any of the studied microsatellites and the PKD1 chromosomes. The absence of linkage disequilibrium in our series suggests that the possibility of a small number of founder chromosomes in our population is remote; thus many different independent mutations are expected in the PKD1 gene. We found a recombination event between D4S423 and D4S1542, which places the PKD2 gene proximal to D4S423, and another recombination between CW2 and AC2.5, both markers being centromeric to the PKD1 gene.

Because genetic heterogeneity was established for ADPKD (14,15,28,42), it has been repeatedly reported that PKD2 is a milder form of the disease, on the basis of the age of onset of ESRD (23,24,26-29,45). When we compared the prevalence of urinary tract infections or urinary calculi in both groups, we found no significant differences, but when we analyzed the age of onset of ESRD, the age of diagnosis of the disease, and the prevalence and age at diagnosis of hypertension, striking differences arose. PKD1 patients were diagnosed 14 yr earlier than PKD2 patients (27.43 versus 41.4 yr) and reached ESRD 19.2 yr earlier than PKD2 patients (53.45 versus 72.71 yr). As other authors have already reported (23,28,29), PKD1 patients have a higher prevalence of hypertension, especially among subjects under 40 yr of age, and are diagnosed earlier with this condition than hypertensive PKD2 patients. The lesser severity of PKD2 patients should be taken into account when counseling PKD2 families; however, it should be noted that there are families with definite linkage to the PKD1 locus that have an age of onset as late as PKD2 families (23,37). We found some examples that support this, such as a PKD1 family with ADPKD members in their 60s and 70s and

<table>
<thead>
<tr>
<th>Category</th>
<th>Age of Subject (yr)</th>
<th>PKD1</th>
<th>PKD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>False Negative</td>
<td>(&lt;30yr) 4/64 (6.2%)</td>
<td>2/6  (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Hepatic Cysts (%)</td>
<td>&lt;20yr 2/35 (5.7%)</td>
<td>0/4  (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21-40yr 28/48 (58.3%)</td>
<td>2/6  (33.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(&gt;40yr 52/58 (89.6%)</td>
<td>10/10 (100%)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic Cysts</td>
<td>5/117 (4.3%)</td>
<td>0/17 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

aADPKD patients whose pancreas was sonographically visualized.

Figure 4. Age of onset of ESRD in parent-offspring pairs. The x axis represents the age of onset of ESRD in parents, and the y axis the age of onset of ESRD in offspring. The dotted line represents the theoretical line for unaltered age of onset of ESRD in parent and offspring. The dashed line leaves below all the pairs with the offspring reaching ESRD more than 10 yr earlier than the parent. Between both lines are the pairs in which the offspring reaches ESRD between 1 and 9 yr earlier than the parent. The different symbols allow us to distinguish between paternal and maternal transmission and between PKD1 and PKD2.

Table 3
Sonographic findings in PKD1 and PKD2 subjects

<table>
<thead>
<tr>
<th>Category</th>
<th>Age of Subject</th>
<th>PKD1</th>
<th>PKD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>False Negative</td>
<td>(&lt;30yr)</td>
<td>4/64 (6.2%)</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>(&gt;40yr)</td>
<td>52/58 (89.6%)</td>
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</tbody>
</table>

aADPKD patients whose pancreas was sonographically visualized.
normal renal function and a PKD2 family with a member entering ESRD at 55 yr of age. That implies that we still must be cautious when establishing prognosis on the basis of genetic linkage and we should consider the other affected members of the family to estimate the ESRD of a given patient.

The diagnosis of ADPKD before the onset of symptoms can conveniently be made by ultrasonography. Although it is less sensitive than linkage analysis, especially among young individuals (6,41,46), we obtained very good results with this technique when it was performed by an experienced renal sonographer. False-negative results in sonography were scarce and presented only in the first or second decade.

We did not find any significant differences of kidney size for PKD1 versus PKD2. We found that the prevalence of hepatic cysts in PKD1 and PKD2 subjects increases with age. No differences between PKD1 and PKD2 as to the prevalence of hepatic cysts were evident, contrary to the findings of Wright et al. (28), who found a higher prevalence of hepatic cysts among PKD2 subjects, and Bogdanova et al. (22), who found it for PKD1. Pancreatic cysts are well referenced in the literature as being one of the possible findings in ADPKD, but their prevalence has not been previously reported (47). The prevalence that we established for PKD1 patients was 4.2%. We did not find any unaffected subject or PKD2 patient with pancreatic cysts. Although we found pancreatic cysts in two members of the same family, there is not enough evidence to define familial aggregation. Familial aggregation has also been suggested for intracranial aneurysms (3,48). Although we have not screened the subjects of this study for cerebral aneurysms, we have found evidence of familial aggregation in two members of two different PKD1 families.

Anticipation has been suggested by Dalgaard, but without definite results (49). More recently, Fick et al. (32) and our group (50) reported the existence of this phenomenon among ADPKD families. In the study presented here, we found anticipation, defined as at least a 10-yr–earlier onset of ESRD in the offspring of any parent–offspring pair, in eight of 35 informative PKD1 families and in two of three informative PKD2 families. Although there could be some bias as a result of the criteria used for anticipation, including a short period of follow-up, the lack of offspring in young patients with an early age of onset of ESRD, or the selection of severe forms of ADPKD, we think that in some families there are reliable data supporting this phenomenon. The presence of anticipation in our ADPKD study confirms this finding, already reported in another clinical population (32), and raises the possibility of a mutation or a modifier gene being responsible for this phenomenon.

Bear et al. (41) presented data suggesting genetic imprinting, with the disease being more severe when it was inherited from the mother than from the father. Fick et al. (32) supported the same argument when analyzing the inheritance of the disease in severely affected children. In our series, as well as in the study of Florijn et al. (51), we did not find evidence of genetic imprinting, either in PKD1 or in PKD2 families. The only evidence of difference in the severity of the disease between sexes has been an earlier age of onset of ESRD in men (3.5 yr for PKD1 and 3 yr for PKD2). This gender effect has been noted in both human and animal ADPKD models (26,52). Dalgaard (49) noted that, at the age of 50 yr, more ADPKD men than women had died. Gretz et al. (53) reported that women entered ESRD on average 6 yr later than men. The reason for the gender difference, more likely than an imprinting effect, is apparently particular physiologic differences between men and women.

At present, the diagnosis of ADPKD depends on ultrasound scanning and linkage analysis. Because genetic heterogeneity is now well established in this disease, linkage must be clearly established in a family before marker data are used for prenatal or presymptomatic diagnosis. Our observation that PKD2 is a milder form of renal disease confirms what already has been pointed out in the literature (14,24,26,29,30,45) and may influence medical practitioners when they are counseling patients who are afflicted with PKD1 or PKD2. Because of the apparently low prevalence of PKD2, multicenter studies should be performed to obtain reliable data for this entity. Although the PKD1 gene has already been cloned (8–10), linkage analysis will remain the best approach for molecular diagnosis of the disease until the mutations in the PKD1 gene are well defined. If, as is expected, the number of different mutations found is high, then linkage analysis will persist as an essential tool in molecular diagnosis. Characterization of ADPKD genes, their mutations, and the proteins they encode should provide an understanding of the molecular pathology and the phenotypic heterogeneity of the disease, and may ultimately facilitate the basis for possible therapeutic interventions.

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To the Right Honourable, right worshipful, whether more or less dignified, who have been or hereafter may be my patients, as also to the courteous or discourteous Reader.

Your Honour, . . . . . . . hath often heard it spoken from the mouth of many a well-read and experienced man in Physick that the urine is an Harlot, or a Lyer, and that there is no certain knowledge of any Disease to be gathered from the Urine alone, nor any safe judgement to be exhibited by the same. You have been (likewise) often told, by physicians, that it were far better for the Physician to see his Patient once, than to view his Urine twenty times.