

## Short reports

## A family with a milder form of adult dominant polycystic kidney disease not linked to the PKD1 (16p) or PKD2 (4q) genes

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### Abstract

**Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disease. Most families show positive linkage to polymorphic markers around the PKD1 (16p13.3) or PKD2 (4q21-23) loci. The PKD1 and PKD2 genes have been cloned and mutations defined in a number of patients. Several clinical studies have described a milder phenotype for PKD2 patients. More recently, evidence for a third genetic locus has been found in one Portuguese, one French-Canadian, and one Italian family.**

**We identified a Spanish family with negative linkage to the PKD1 and the PKD2 loci. This family showed a very mild clinical phenotype compared to the other forms of ADPKD, including the non-PKD1/non-PKD2 families previously described.**

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Autosomal dominant polycystic kidney disease (ADPKD) has a prevalence of about 1 in 1000. It accounts for approximately 10% of cases of end stage renal failure. ADPKD is characterised by cyst formation in the kidney and may occur at any time of life. Other abnormalities, mainly hypertension and liver cysts, are also frequently found in ADPKD patients. Renal cysts develop with a probability that increases with age and can be detected by ultrasonography.<sup>1</sup>

Linkage analysis on affected families has shown the existence of at least three loci involved in this disease. Most of the families showed positive linkage for markers around the PKD1 gene on 16p13.3.<sup>2,3</sup> The PKD1 gene has been cloned and mutations have been found in several families.<sup>4-7</sup> A second ADPKD locus, designated PKD2, resides on chromosome 4q21-23.<sup>8,9</sup> The PKD2 gene has recently been cloned and mutations in this gene, responsible for a less frequent form of the dis-

ease, have been found in a number of families.<sup>10</sup> Evidence for at least a third locus has recently been found in families showing negative linkage to the 16p and 4q markers.<sup>11-14</sup>

Several studies have shown that the genetic heterogeneity that characterises ADPKD has a phenotypic correspondence, with a milder phenotype in the non-PKD1 forms. Cysts are apparently detected at an older age in those patients with the non-PKD1 forms of the disease than in those with the classical ADPKD1 form.<sup>15-17</sup>

In studying the genetic heterogeneity in the Spanish population, we identified a family that showed negative linkage to the PKD1 and the PKD2 loci. In this family, patients showed an atypical, very mild presentation of ADPKD. We therefore suggest that this form of the disease could affect more families than suggested, but patients in most of them do not develop the typical symptoms of ADPKD.

### Materials and methods

#### FAMILY DATA

Members of a two generation family consisting of both parents and five children (fig 1) were ultrasonographically evaluated, and the diagnosis of ADPKD was based on the finding of at least one cyst in one kidney and two or more cysts in the other. The family, with a negative family history for ADPKD, was screened in the Nephrology Department, Hospital Central de Asturias, Oviedo, Spain. Subject 7, 36 years old, had congenital posthydronephrotic atrophy in the left kidney. Hypertension in this patient was diagnosed at the age of 27 years. Subject 1, 69 years old, suffered from mild hypertension diagnosed at the age of 67 years. Subjects 2, 3, 4, 5, and 6 (65, 40, 42, 44, and 38 years old, respectively) were healthy, and showed no sign of hypertension.

Ultrasonographic analysis showed multiple cortical cysts in both kidneys in subjects 1, 5, and 7. All cysts were small, 1-2 cm in diameter. Subjects 7 and 5 also had two and three small liver cysts, respectively.

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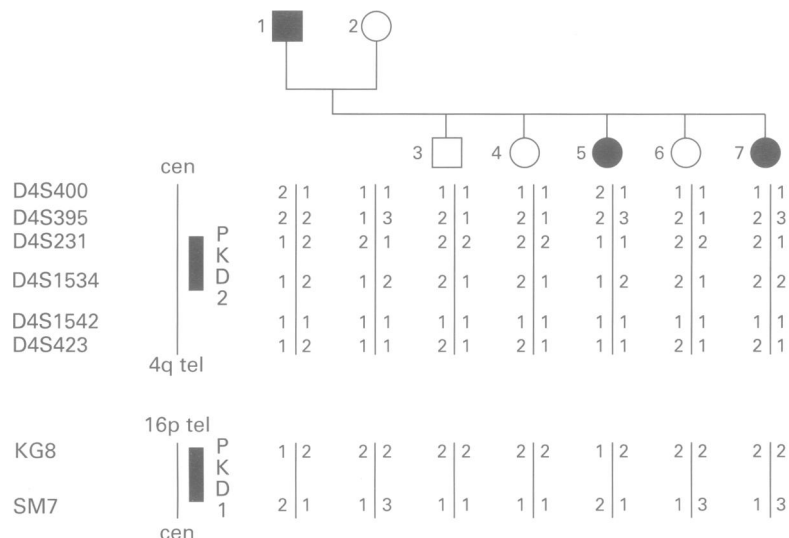


Figure 1 Haplotypes defined by markers closely linked to the PKD2 (4q) and PKD1 (16p) genes.

#### DNA MICROSATELLITE ANALYSIS

DNA was obtained from each person. Two 16p microsatellite polymorphisms (SM7 and KG8) were studied.<sup>2-4</sup> KG8 is located at the 3' end of the PKD1 gene. Six microsatellites on 4q21 (D4S1542, D4S395, D4S400, D4S231, D4S423, and D4S1534) flanking the PKD2 locus were also analysed.<sup>8,9</sup> PCR microsatellite analysis was done as previously described.<sup>15</sup>

#### ANALYSIS OF THE *AVAII* RFLP IN THE PKD1 GENE

In a search for mutations in the PKD1 gene in 13 families, we detected a SSCP variant in one patient. The electrophoretic mobility shift resulted from a C to A change at codon 4058 (GCC:AlaGTC:Val). This mutation created an *AvaII* site and was also found in healthy donors from the same population. A sequence of 456 bp including nucleotides 12236 (exon 44) to 12609 (exon 45) and intron 44 (83 bp) was PCR amplified with primers CGCCAGTGGTCCGTCTTTG and AGGAACAAC-TCCACCATCACGTAG.<sup>6</sup> PCR consisted of 0.5 µg of genomic DNA, 30 pmol of each primer, 2.0 mmol/l MgCl<sub>2</sub>, 1 × *Taq* buffer, and 1 U of *Taq* DNA polymerase (Promega) in a final volume of 30 µl. After 30 cycles of 98°C for 30 seconds, 66°C for one minute, and 72°C for 45 seconds, 10 µl of reactions containing a single fragment of the appropriate size were digested with 10 U of *AvaII* and electrophoresed on a 2% agarose gel. Chromosomes carrying the C to A mutation were digested, giving two fragments of 231 and 225 bp.

#### Results

The proband, a 36 year old female, suffered from hypertension attributed to atrophy of the left kidney. There was no previous family history of any inherited kidney disorder, including ADPKD. More recently, mild hypertension was diagnosed in the father at the age of 67 years. We then performed ultrasonographic analysis on all family members, and multiple small bilateral cysts were detected in three people, including the father, the proband, and one of her sisters (subjects 1, 5, and 7, fig 1).

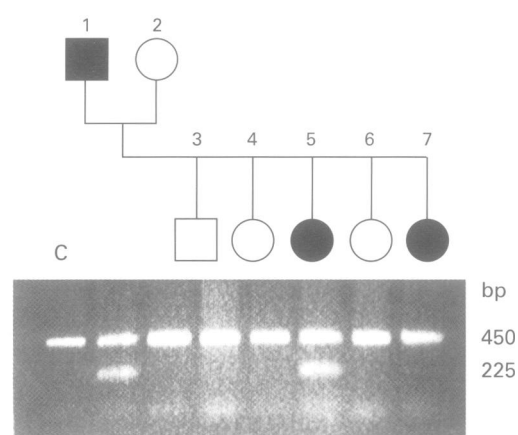


Figure 2 Segregation of the *AvaII* polymorphism intragenic to PKD1. The C to A change at codon 4058 introduced an *AvaII* site, giving two fragments of 231 and 225 bp seen as a single band on agarose gels.

The family was finally diagnosed as having a mild form of ADPKD. Linkage analysis showed that both sisters had inherited different 16p and 4q haplotypes from the father (fig 1). Recombination was excluded because these markers were closely linked to and flanked the PKD1 and PKD2 genes.

In a search for mutations in the PKD1 gene in affected families we identified a C to A mutation at codon 4058. This is a missense mutation (A4058V) that creates an *AvaII* site. Analysis of 50 healthy donors showed that 10% carried the mutation. The family described here was informative for the *AvaII* polymorphism, also showing a negative linkage to the PKD1 locus (fig 2).

#### Discussion

In a previous study we analysed 17 families with a classical clinical presentation of ADPKD. Positive linkage to PKD1 was found in 12 of the 17 families. The five non-PKD1 families had positive values for linkage to the 4q21 markers. Analysis of patients from these families showed that the ADPKD2 form had a milder phenotype, with hypertension, cysts, and end stage renal disease developing less frequently and later in life.<sup>17</sup>

Here we have described a family with negative linkage to 16p and 4q markers, including a new polymorphism in the PKD1 gene also found in an Italian ADPKD1 family.<sup>18</sup> The father and two children showed the features that characterise ADPKD, including multiple cysts in both kidneys. The two affected children inherited different 16p and 4q haplotypes from the affected father. Thus, we concluded that the multiple kidney cysts resulted from a mutation at a third locus. The frequency of non-PKD1/non-PKD2 families is very low and only four families have been described world wide.<sup>11-14</sup> We cannot determine whether ADPKD in these families is the result of different mutations in the same gene or if more than one alternative gene exists.

Finally, the extremely mild phenotype observed in our family could imply that a number of families are never diagnosed as having ADPKD. In this case, the frequency of

non-PKD1/non-PKD2 families would be higher than the current data indicate.

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