

DNA microsatellite analysis of families with autosomal dominant polycystic kidney disease types 1 and 2: evaluation of clinical heterogeneity between both forms of the disease

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Abstract

We studied 17 large families affected by adult dominant polycystic kidney disease (ADPKD). Ultrasonographic analysis was performed on all the family members. DNA microsatellite markers closely linked to PKD1 on 16p13.3 were analysed, and linkage of the disease to this locus was determined. Families showing a negative linkage value were evaluated for linkage to the PKD2 locus on 4q.

Five of the 17 families showed negative linkage for the 16p13.3 markers. In these families significant linkage to 4q was obtained. Renal cysts developed at an earlier age in PKD1 mutation carriers, and end stage renal failure occurred at an older age in people affected with PKD2.

Analysis of large families with ADPKD in a Spanish population indicates that this is a genetically heterogeneous disorder, but mutations at only two loci are responsible for the development of the disease in most if not all the families. Clinicopathological differences between both forms of the disease occur, with subjects with ADPKD2 having a better prognosis than those with mutations at PKD1.

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Autosomal dominant polycystic kidney disease (ADPKD) has a prevalence of about 1 in 1000, accounting for 8 to 10% of cases of end stage renal failure. ADPKD may occur at any time in life, and is characterised by cyst formation in the kidney and, to a lesser extent, in the liver. Other gastrointestinal and cardiovascular abnormalities are often found in ADPKD patients. Renal cysts are detected by ultrasonography, with a probability that increases with age. Several studies have described the presence of cysts in most mutation carriers by the age of 30 years, while some of the carriers younger than 30 years remain asymptomatic.^{1,2}

Linkage analysis on affected families showed the existence of at least two loci involved in this disease. About 90% of the families showed positive linkage to markers on 16p13.3.³⁻⁶ The PKD1 gene on 16p has recently been isolated and partially characterised.⁷ Mutations at PKD1 define the classical form of the disease, also known as ADPKD1. Several DNA poly-

morphic markers flanking PKD1 have been described.⁸⁻¹⁰ A second ADPKD locus has recently been mapped to 4q21.^{11,12} Microsatellites flanking this second locus, designated PKD2, have been described. Mutations in the PKD2 gene are responsible for an alternative, less frequent, form of the disease, designated ADPKD2.^{13,14} Several studies have suggested that the genetic heterogeneity that characterises ADPKD has corresponding phenotypic heterogeneity. Cysts are apparently detected at an older age in those patients having the alternative form of the disease than in those who have the classical ADPKD1 form.^{15,16}

We have analysed 17 large Spanish families with several microsatellite markers around PKD1 and PKD2 in an attempt to define the linkage of the disease to these loci, the existence of further genetic heterogeneity, and to compare the clinical course of ADPKD1 and ADPKD2 patients.

Methods

FAMILIES

Seventeen large families affected by ADPKD were studied. Family members were ultrasonographically evaluated, with symptomatic affected subjects showing at least one cyst in one kidney and at least two cysts in the other kidney. A family was considered to be large (for linkage analysis purposes) when at least three affected members and two unaffected children (older than 30 years) of an affected member were available. This minimum criterion was established by the European Concerted Action on ADPKD. A total of 188 members of the 17 families had an affected parent, thus being at risk of having ADPKD. All family members at risk of having ADPKD were ultrasonographically evaluated during 1993.

DNA MICROSATELLITE ANALYSIS

DNA was obtained from each subject. Four microsatellite polymorphisms ((CA)_n repeats) around the PKD1 gene on 16p13.3 were studied (fig 1). AC2.5 (D16S291)¹⁷ and SM7 (D16S284)⁹ have a proximal location relative to PKD1 (fig 1). KG8 is located intragenic, at the 3' end of the PKD1 gene.⁷ D16S521 is a subtelomeric microsatellite.⁸ Four microsatellites on 4q21 (D4S395, D4S414, D4S423,

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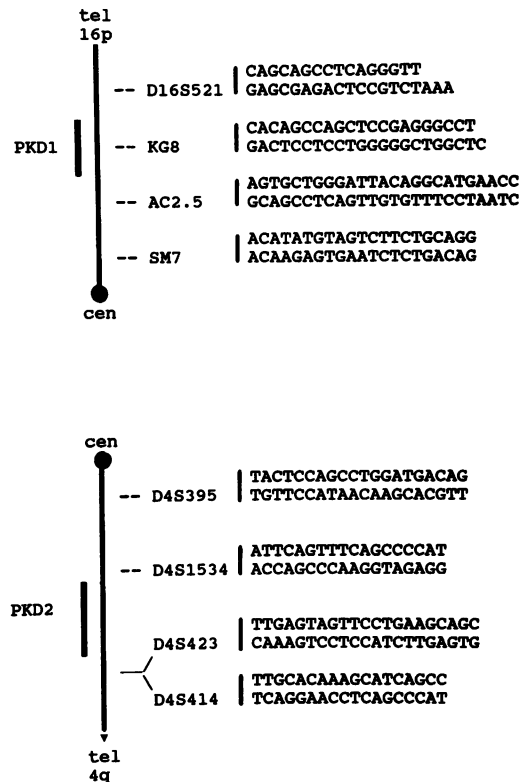


Figure 1 Map of the microsatellite markers around the PKD1 (16p13.3) and PKD2 (4q21) genes.

and D4S1534) were analysed in those families that showed negative linkage to the 16p markers^{11,12} (fig 1).

PCR consisted of 100 ng of genomic DNA in a final volume of 10 μ l containing 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.5), 200 μ mol/l of each dATP, dGTP, and dTTP, 100 μ mol/l of dCTP, 0.1 μ Ci of ³²P-dCTP (3000 Ci/mmol, Amersham), 10 pmol of each primer (fig 1), 2.0 mmol/l MgCl₂, and 1 unit of *Taq* polymerase (Promega). After an initial denaturing step (three minutes, 98°C) the microsatellite sequence was amplified by 30 cycles of 98°C for 30 seconds, the annealing temperature for 30 seconds, and an extension of 72°C for 30 seconds. A final extension step for five minutes at 72°C was performed. Annealing temperature was 59°C except for AC2.5 which was annealed at 65°C.

Ten μ l of formamide loading buffer (95% formamide, xylene cyanol) were mixed with the PCR reaction and this mixture was heated at 98°C for five minutes. Five μ l were loaded onto a 40 cm long 6% denaturing sequencing gel (acrylamide 5.7%, bis-acrylamide 0.3%, urea 45%). Gels were run with TBE buffer (Tris-HCl 90 mmol/l, boric acid 90 mmol/l, EDTA 2.5 mmol/l) under denaturing conditions, vacuum dried, and autoradiographed for two hours to one day.

LINKAGE

Linkage analyses were performed on the 17 families. The decimal logarithm of the odds ratio for linkage (lod score, Z) was obtained for different recombination frequencies (θ) using the LINKAGE program.^{18,19} Fisher's

exact test was used to compare the age at cyst development, the age at onset of end stage renal failure, and the incidence of hypertension in subjects with ADPKD1 and those with ADPKD2.

Results

Positive linkage to 16p13.3 markers was found in 12 of the 17 families (table 1). Because the AC2.5, SM7, or KG8 microsatellites were informative in all the families, and these loci did not recombine with PKD1, the maximum lod score values were obtained at the null recombination fraction, with the lowest lod score in any family estimated as being 1.35. This value represents a probability in favour of linkage of 44 to 1. Eight different AC2.5-SM7-KG8 haplotypes were found in the 12 PKD1 families. A negative lod score for 16p13 was obtained in five of the families. These non-PKD1 families had positive values for linkage to the 4q21 markers. Haplotypes defined by the 4q loci were constructed, and the lowest lod score for linkage was estimated as being 1.57, representing a probability in favour of the 4q linkage of 37 to 1 (table 1). Examples of ADPKD1 and ADPKD2 families are shown in fig 2. In one of the ADPKD2 families a recombination located the PKD2 locus between D4S1534 and D4S414 (fig 2).

A total of 77 people in the 12 ADPKD1 families were carriers of the disease (DNA analysis). Sixty-one of these were older than 21 years and all of them showed ultrasonographic renal cysts. Twenty five of the ADPKD1 affected subjects had end stage renal disease, with the earliest age at onset at 32 years (mean age 47.6, SD 10.3). A total of 36 subjects in the five ADPKD2 families were carriers of the disease, and cysts were also present in all carriers older than 21 years (30 people). Six ADPKD2 patients had end stage renal disease, with the earliest age at onset at 44 years (mean age 56.3 years, SD 7).

Table 2 summarises the data on the age of mutation carriers and the presence of cysts, the age at onset of end stage renal disease, and the incidence of hypertension in our patients. Comparison of these clinical parameters between ADPKD1 and ADPKD2 patients

Table 1 At risk subjects, number of mutation carriers, and maximum lod score values for each of the 12 ADPKD1 and five ADPKD2 kindreds

	At risk members	Mutation carriers	Linkage analysis (marker/Z _{max} (θ))
<i>ADPKD1 families</i>			
1	15	10	AC2.5/2.89 (0.0)
2	12	6	AC2.5/1.90 (0.0)
3	8	6	D16S521/2.10 (0.0)
4	8	6	SM7/1.98 (0.0)
5	7	4	SM7/1.65 (0.0)
6	11	5	AC2.5/1.35 (0.0)
7	9	3	KG8/1.75 (0.0)
8	6	5	AC2.5/1.81 (0.0)
9	13	8	AC2.5/2.25 (0.0)
10	7	5	SM7/1.80 (0.0)
11	19	12	AC2.5/3.36 (0.0)
12	7	7	SM7/1.80 (0.0)
<i>ADPKD2 families</i>			
13	21	12	D4S1534/4.43 (0.0)
14	16	6	D4S414/2.06 (0.0)
15	16	8	D4S1534/2.57 (0.0)
16	10	5	D4S1534/1.98 (0.0)
17	9	5	D4S395/1.57 (0.0)

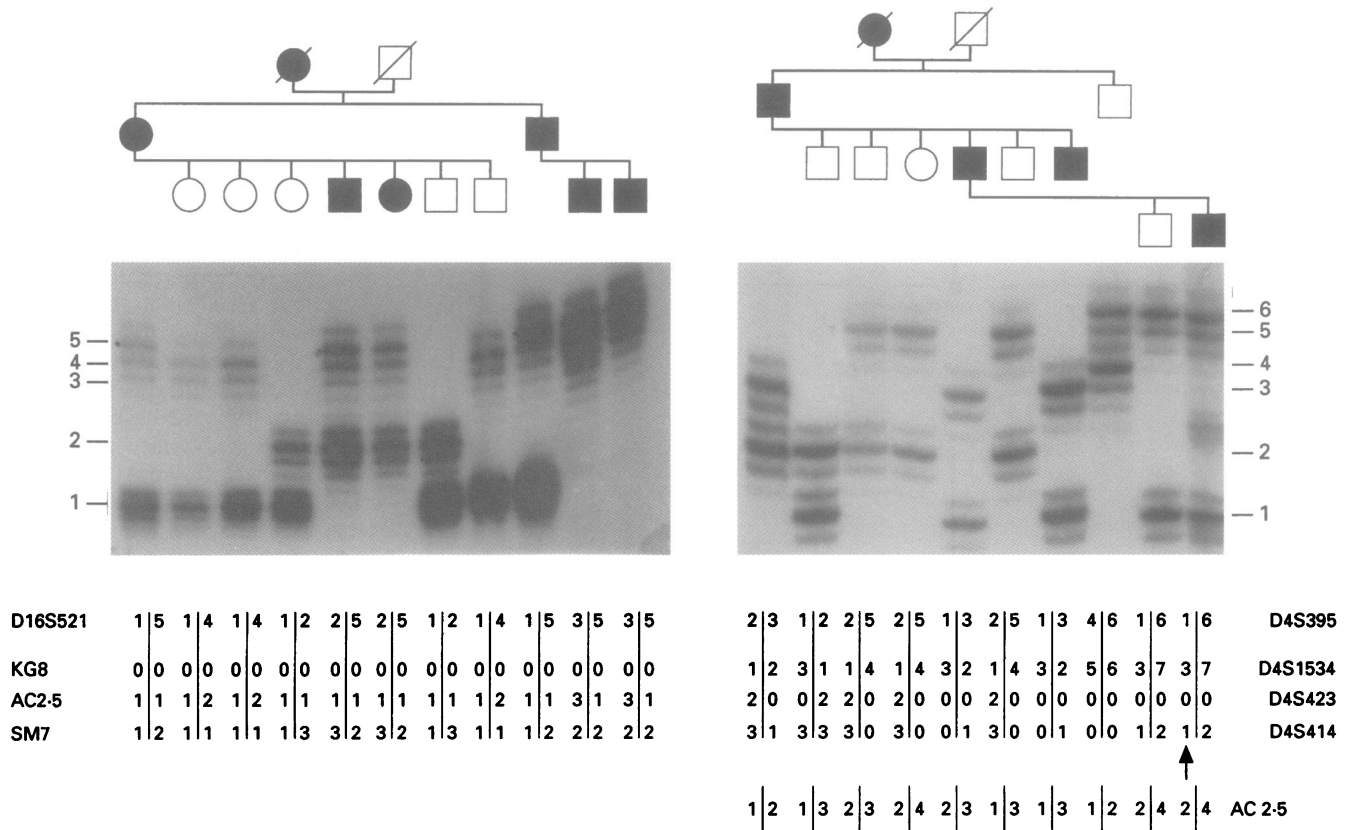


Figure 2 Microsatellite analysis of one ADPKD1 family (left) and one ADPKD2 family (right). Arrow indicates a recombinant chromosome that maps PKD2 between D4S1534 and D4S423.

showed significant differences. Only three out of the 16 (18.75%) PKD1 mutation carriers younger than 20 years were free of renal cysts, compared to five out of the six (83.3%) PKD2 mutation carriers in the same age group. The difference between both groups of patients was statistically significant ($p < 0.01$). When considering patients with renal cysts who are therefore at risk of developing secondary renal insufficiency, a difference between both groups was also found. Only 19% (6/31) of the ADPKD2 patients compared to 36% (27/74) of the ADPKD1 patients needed substitutive therapy. The difference was significant for the 31 to 40 year group, where no ADPKD2 patient (0/12) and 57% (8/14) of the ADPKD1 patients had developed renal insufficiency ($p < 0.002$).

Table 2 Comparative incidences of ecographic cysts (cysts-), end stage renal disease (ESRD), and hypertension in genetic carriers (DNA+)

Age (y)	ADPKD1	ADPKD2	p*
	Cyst-/DNA+		
0-10	1/1	1/1	NS
11-20	2/15	4/5	0.014
21-30	0/19	0/6	NS
>31	0/42	0/24	NS
	ESRD/DNA+		
21-30	0/19	0/6	NS
31-40	8/14	0/12	0.002
41-50	9/19	1/3	NS
>51	8/9	5/9	NS
	Hypertension/DNA+		
0-30	3/35	1/12	NS
31-40	8/14	1/12	0.012
41-50	10/19	2/3	NS
>51	8/9	3/9	0.003

NS = not significant.

Discussion

Diagnosis in presymptomatic ADPKD carriers is frequently made by ultrasonography. However, false negative ultrasonographic diagnosis has been described.²⁰ In a previous study on our population we found that 22% of PKD1 mutation carriers younger than 30 years did not show renal cysts.²¹ This age dependence of manifestation of symptoms prompted several groups to develop DNA based protocols for family studies.

A different clinical course has been described for ADPKD, with patients having the classical form (ADPKD1) showing renal cysts at an earlier age than those patients having mutations in a gene other than PKD1.^{15,16} Previous studies on this topic have compared the classical form with a non-classical form, but the significance of these studies has been complicated by the lack of information on the genetic nature of the non-PKD1 form or forms of polycystic kidney disease. In our study comparison of both groups of patients showed significant differences, both in the age of cyst development and in the age of onset of end stage renal disease. The late development of renal cysts in ADPKD2 patients must be taken into account when performing echographical screening in these at risk subjects. This technique has to be considered as unreliable until later stages of life, and DNA polymorphic markers should be used as a screening tool in at risk ADPKD2 subjects younger than 30 years. The earlier development of renal cysts in ADPKD1 patients produces a faster deterioration of renal function and an earlier start

of renal substitutive therapy. Hypertension was more frequent among ADPKD1 patients, confirming the previously suggested difference between the two forms of the disease.²²

Finally, eight different 16p haplotypes were found in the 12 ADPKD1 families. To date only three mutations at PKD1 have been identified.⁷ If ADPKD1 is the result of many different mutations, a direct approach for genetic counselling would be difficult and microsatellite analysis could be necessary for family studies.

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