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Genetic Heterogeneity of *PKD1* and *PKD2* Genes in Iran and Determination of the Genotype/Phenotype Correlations in Several Families with Autosomal Dominant Polycystic Kidney Disease

Ramin Radpour[*1 & 2], Mina Rezaee[1], Mahdi M. Haghighi[1], Mina Ohadi[1], Hossein Najmabadi[1] and Asghar Hajibeigi[1]

[1]*Genetic Research Center of Social Welfare and Rehabilitation Sciences University;* [2]*Dept. of Genetics and PGD, Reproductive Biomedicine Research Center of Royan Institute, Tehran, Iran*

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic nephropathy, which is characterized by replacement of renal parenchyma with multiple cysts. In Iran, the disease prevalence within the chronic hemodialysis patient population is approximately 8-10%. So far, three genetic loci have been identified to be responsible for ADPKD. Little information is available concerning the pattern of linkage in Iranian population. In the present study, the linkage analysis was performed using three pairs of polymorphic microsatellite markers including 16AC2.5-CA (D16S291), SM7-CA (D16S283) and KG8-CA (intragenic marker at the 3' end of the gene). These markers are closely linked to the ADPKD1 locus and three pairs of the selected polymorphic microsatellite markers including YUNCA9 (D4S231), AFM155xe11 (D4S1534) and AFM224x6 (D4S423), which were closely linked to the ADPKD2 locus. In parallel, the genomic DNA of 150 unrelated healthy individuals was used to determine frequency, heterozygosity rate and polymorphic information content (PIC) for each marker. In our study, haplotypes were constructed in a number of ADPKD families using respective markers. Assignment of the disease gene loci was performed following phasing and haplotype construction, genotype/phenotype correlations were deduced from the constructed haplotypes. Analysis of clinical data confirms a milder ADPKD phenotype for *PKD2* families in Iran. Our results showed relatively high heterozygosity rates and PIC values for some markers, while the most informative markers were KG8 (PIC: 0.772) and 16AC2.5 (PIC: 0.689) for *PKD1* gene and AFM224x6 (PIC: 0.712) for *PKD2* gene. We report here the first molecular genetic study of ADPKD and the existence of locus heterogeneity for ADPKD in Iranian population by performing linkage analysis on 15 affected families. Eleven families showed linkage to *PKD1* and two families linked to *PKD2* gene. In 2 families, *PKD1* markers were common in all affected members but *PKD2* markers were not informative. *Iran. Biomed. J. 10 (1): 1-8, 2006*

Keywords: Autosomal dominant polycystic kidney disease (ADPKD), Microsatellite marker, Genetic diagnosis, Linkage analysis

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary systemic nephropathy (polycystic kidney disease [MIM 173900]), characterized by the development of cysts in the ductus organs (mainly the kidneys and the liver), also with gastrointestinal and cardiovascular abnormalities [1]. The kidney cysts gradually enlarge and their numbers grow, destroying in this way the functional parenchyma. As a consequence, the patients develop chronic renal failure (CRF), which progresses to end-stage chronic renal failure (ESCRF), requiring substitutional therapy (hemodialysis or kidney transplantation). ADPKD patients may benefit if the diagnosis can be established before symptoms develop [2].

ADPKD is one of the most common genetic disorders. The gene frequency in the general population has been estimated to be about 1 in 400-1,000 in the Western countries [3, 4] and is likely to be similar in Iran. ADPKD is genetically heterogeneous and there are at least three possible

*Corresponding Author; E-mail: rradpour@royaninstitute.org

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loci involved in this disease [5]. Mutations of the polycystic kidney disease 1 gene (*PKD1* [MIM 601313]) on chromosome 16 p13.3 are the most common and account for about 85% of all cases of ADPKD [6]. The *PKD1* gene has been cloned, and the structure of its encoded complex protein, called polycystin, was determined [7]. *PKD2* [MIM 173910]) is located on chromosome 4q21-23 [8]. It is defective in most of the remaining ADPKD families [6-8]. A small number of families are affected by one or more undetermined loci (*PKD3* [MIM 600666]), which do not map to the above loci.

Direct screening for *PKD1* mutations in ADPKD is difficult due to the large gene size and extensive homology of *PKD1* to other parts of the human genome [9]. The *PKD2* gene is more amenable because of its smaller size and absence of extensive homology to other parts of chromosome 4. However, no "hot-spot" mutation has been identified in the *PKD1* or *PKD2* genes [10]. Genetic diagnosis through screening for defects in the *PKD1* and *PKD2* is technically possible but the cost and times involved are prohibitive and severely limit its clinical application. Alternative ways that identify the transmission of ADPKD are needed. In this communication, we report our finding of using DNA microsatellite marker analysis to determine the transmission of ADPKD in Iranian families.

MATERIALS AND METHODS

Normal population. Genomic DNA of 150 unrelated healthy individuals from Iran was obtained. Based on those samples, we determined the allele frequencies, heterozygosity rate, and polymorphic information content (PIC) values of the six microsatellite markers in our local population.

ADPKD families and clinical evaluation. Several families with the history of hereditary renal cysts were recruited and informed consent was obtained before our investigation. The following criteria were used for diagnosis of ADPKD: (i) the presence of at least two renal cysts (unilateral or bilateral) in an at-risk individual aged <30 years; (ii) the presence of at least two renal cysts in each kidney in an at-risk individual aged 30-59 years; (iii) the presence of at least four renal cysts in each kidney in an at-risk individual aged ≥60 years. Abdominal ultrasonography and full biochemical studies were arranged, that included serum urea, creatinine, uric acid, urine microscopy, and a renal ultrasound. Urine studies for fractional extraction of sodium, fractional excretion of uric acid, and protein/ creatinine ratio were also performed. Twenty-four hour urine collections for total protein excretion were performed in those with protein-urine on urine analysis studies. Creatinine clearance was calculated according to sex, age, lean body mass (LBM), and serum creatinine based on the equation (140 age) × LBM/72 × serum creatinine for males, or (140 age) × LBM/72 × serum creatinine × 85% for females and was corrected for a body surface area of 1.73 m^2. Following their clinical assessments by nephrologists, the blood samples of each patient and first-degree relatives were collected for DNA extraction. A total of 4 affected kindred were included in this study following informed consist. A detailed family history obtained from each subject. For some of the cases included here, since they died many years ago, there are no accurate clinical data and their children provided the information that they suffered from renal failure.

Controls. Controls were spouses of those affected in each family as well as unaffected siblings of individuals with *PKD1* or *PKD2* (normal renal imaging and linkage results showed that their genetic risk was less than 1%). Affected individuals were matched with their partners whenever possible, irrespective of whether they were alive or dead. Non-affected siblings who had died were included as controls only if there was proof that they had not ADPKD.

PKD1 and PKD2 linkage studies and haplotype construction. Large ADPKD families, selected only because of their suitability for study by genetic linkage, were tested for evidence of linkage to the *PKD1* and *PKD2* loci by means of polymorphisms flanking both loci. Two-point linkage analysis was performed and LOD scores were calculated by the FASTLINK suite of programs [11]. Data available from the linked markers for *PKDI* and *PKD2*, used in each family were combined by means of a Bayesian weighting formula'5 to estimate the likelihood that a family shows linkage to one or other locus. Marker-allele frequencies were obtained from married-in individuals and from reconstruction of founder genotypes. The locations of the markers relative to *PKD1* and *PKD2* are shown in Figure 1, respectively. The haplotypes of these microsatellite markers in members of the ADPKD families were determined.

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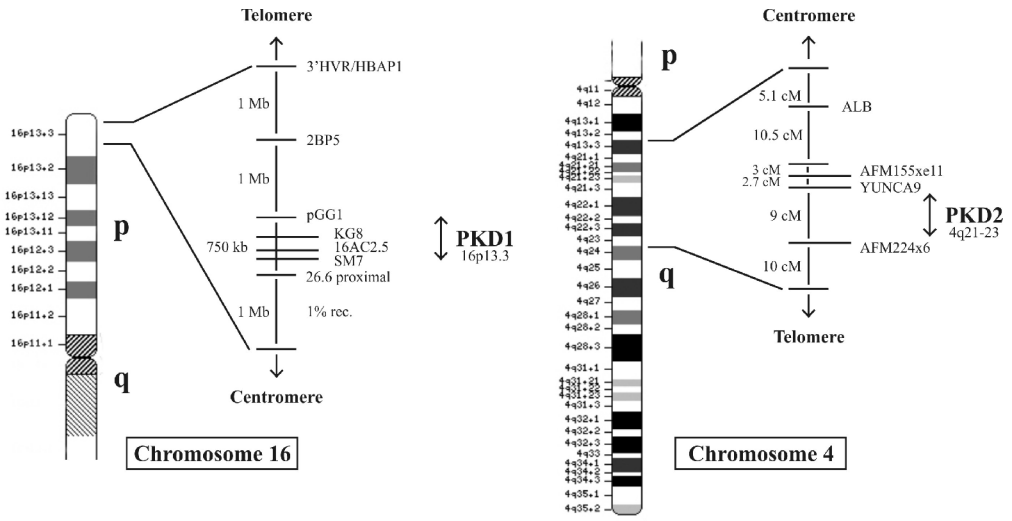

Fig. 1. Genomic locations of the micro satellite markers relative to *PKD1* on chromosome 16 and *PKD2* on chromosome 4.

Microsatellite variant repeat PCR. Three microsatellite markers KG8 [12], 16AC2.5 [12] and SM7 [13, 14] for *PKD1* gene, YUNCA9 [15], AFM155xe11 [16, 17] and AFM224x6 [17, 18] for *PKD2* gene were amplified by PCR using specific primers. The following is a brief description of the procedure we used. Genomic DNA (30 ng) was PCR amplified in a final volume of 12.5 µl containing, 1 × Cetus buffer II (PE Applied Biosystems, Warrington, UK), 1.5 mmol/lit $MgCl_2$, 200 mol/lit of each dATP, dGTP, dCTP and dTTP, 3 pmol of each primer and one unit of Amplitaq Gold (Applied Biosystems). Amplification condition was an initial denaturing step (6 min for 95°C), followed by 29 cycles of 94°C for 60 seconds, the primer specific annealing temperature (T_a) for 60 seconds and 72°C for 60 seconds. A final extension step for 60 seconds at T_a and 10 minutes at 72°C was performed. The T_a was 69°C for KG8, 63°C for 16AC2.5, 57°C for SM7 and 55°C for *PKD2* markers (D4S1534, D4S231 and D4S423). Polyacrylamide gel electrophoresis was used to resolve the markers according to the molecular size. Denatured PCR product (5 ml) was loaded onto a 12% denaturing polyacrylamide gel. The gel was run in Tris-Borate-EDTA buffer at 35 watts until the xylene cyanol dye reached approxi-mately 5 cm from the bottom of the gel. It was colored by silver nitrate. The gel was then developed and kept for permanent record.

RESULTS

Population studies. We will concentrate mainly on the polymorphic microsatellite repeats closely linked to *PKD1* and *PKD2* genes. Before the analysis of linkage in our population, we characterized the number of alleles, their sizes, PIC and heterozygosity content of the microsatellite markers in 150 unrelated individuals in Iranian population (Table 1). All the microsatellites except SM7 and AFM155xe11 had high heterozygosity rates and were suitable for linkage analysis of ADPKD in Iran.

Table 1. No. of alleles, heterozygosity rates and PIC values of 6 microsatellite markers for linkage analysis of ADPKD in the Iranian population rate.

	Marker	Locus symbol	Maker type	Allele size	Allele no.	Heterozygosity (h)	PIC
PKD1	KG8	N/A	(AC) n	116-130	6	0.80	0.772
	16AC2.5	D16S291	(AC) n	154-170	5	0.73	0.686
	SM7	D16S283	(AC) n	81-107	4	0.68	0.624
PKD2	AFM155xe11	D4S1534	(AC) n	146-158	4	0.60	0.519
	YUNCA9	D4S231	(AC) n	154-169	4	0.64	0.570
	AFM224x6	D4S423	(AC) n	103-125	5	0.76	0.712

PIC, polymorphic information content.

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Table 2. Two-point linkage LOD scores for linkage between ADPKD families and markers close to the *PKD1* gene.

	Recombination fraction				θ	Z	
Family	**0.0**	**0.01**	**0.05**	**0.1**	**max**	**max**	
1203	1.51	1.49	1.41	1.30	0.0	1.51	ADPKD
1208	1.25	1.23	1.11	0.97	0.0	1.25	vs
1214	-infin	-1.57	-0.88	-0.59	>0.5	0.08	16AC2.5
Total	-infin	1.15	1.64	1.68			
1203	2.30	2.26	2.08	1.84	0.0	2.3	ADPKD
1208	1.25	1.23	1.11	0.97	0.0	1.25	vs
1214	0.01	0.01	-0.02	-0.04	0.0	0.01	KG8
Total	3.56	3.5	3.17	2.77			
1203	1.44	1.41	1.28	1.12	0.0	1.44	ADPKD
1208	-2.51	-0.92	-0.30	-0.08	>0.5	0.35	vs
1214	0.81	0.79	0.73	0.65	0.0	0.81	SM7
Total	-0.26	1.28	1.71	1.69			

Linkage analysis. Two-point "maximum likelihood" linkage analysis was performed using the FASTLINK suite of programs. We used a dominant model with a disease gene frequency for *PKD1* and *PKD2* genes. Allele frequencies were calculated from the alleles observed in married-in individuals from the pedigree using PEDMAN-EGER, a computer package developed at the Whitehead Institute (Reeve-Daly, unpublished). LOD scores were calculated at the known recombination fractions between markers and either *PKD1* (Table 2) or *PKD2* (Table 3) locus. Linkage analysis in 15 affected families with microsatellite markers close to *PKD1* and *PKD2* genes, showed the existence of locus heterogeneity in our population.

Family studies and haplotype constructions. Haplotype construction was performed at both *PKD1* and *PKD2* locus. Three microsatellite markers were identified at the *PKD1* locus including 16AC2.5-CA (D16S291), SM7-CA (D16S283) and KG8-CA that were closely linked to *PKD1* locus and three pairs of selected polymorphic markers including YUNCA9 (D4S231), AFM-155xe11 (D4S1534) and AFM224x6 (D4S423) which were closely linked to the *PKD2* locus. Haplotype analysis reveled that each family had *PKD1*- or *PKD2*-linked disease with a unique disease-associated haplotype. Our molecular study of 15 Iranian affected families was suggested that 11 of the 15 families linked to *PKD1* gene whereas 2 families were linked to *PKD2* gene. In 2 families, *PKD1* markers were common in all affected members but *PKD2* markers were not informative.

Clinical findings and genotype/phenotype correlation. A total of 135 subjects from 15 Iranian ADPKD families were studied for the purpose of this report. The total number of patients originating from linked to *PKD1* was 68 and that of patients from *PKD2* families was 25. Totally, 93 investigated subjects were affected (ADPKD1 or ADPKD2), 31 blood relatives were unaffected (at risk subjects), and 11 were spouses. In the *PKD1* families, all members 30 years of age with affected relatives and a *PKD1* associated haplotype had detectable cysts (Table 4). No member entered end-stage renal disease. (ESRD) below the age of 40. Seventeen subjects developed CRF at mean age of 48 years. However, members without the associated *PKD1* haplotype did not enter ESRD before age of 60 year. Haplotype analysis revealed the presence of a unique disease-associated haplotype in each family. Interfamily differences were detected when comparing overall survival. Family 1206 had the shortest survival, with a median age of 47.5 years, and family 1213 had the longest, with a median age of 77.4 years. For renal survival, the difference between families was highly significant (P<0.05).

Table 3. Two-point linkage LOD scores for linkage between ADPKD families and markers flanking the *PKD2* gene in family 1214.

Marker	Recombination fraction				θ	Z
	0.0	**0.01**	**0.05**	**0.1**	**max**	**max**
D4S231	1.52	1.49	1.36	1.19	0.0	1.52
D4S1534	0.95	0.93	0.84	0.73	0.0	0.95
D4S423	1.52	1.49	1.36	1.19	0.0	1.52

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Table 4. Clinical findings and specific characteristics of Iranian ADPKD patients.

Manifestation	Frequency
External	
Gastrointestinal	
o Hepatic cysts	Approximately 53%
o Pancreatic cysts	Approximately 11%
o Colonic diverticula	80% of patients with ESRD
Cardiovascular	
o Cardiac valvular abnormalities	27%
o Intracranial aneurysms	8%
Genital	
o Ovarian cysts	Unknown
o Testicular cysts	Unknown
o Seminal-vesicle cysts	Unknown
Renal	
Anatomic	
o Renal cysts	100%
o Renal adenomas	27%
o Cyst calcification	97%
Hormonal alternations	
o Increased rennin production	Approximately all affected hypertensive adults
o Preserved erythropoietin production	All patients with ESRD
Complications	
o Hypertension	87% (most of patients with ESRD)
o Hematuria and / or hemorrhage	57%
o Acute and chronic pain	75%
o Urinary tract infection	Common
o Nephromegaly	100%
o Renal failure	47% of patients by age >50

ESRD, end-stage renal disease.

PKD2 patients, compared with those who have *PKD1*, present with symptoms later in life, live longer, have a lower risk of progressing to renal failure, and have fewer complications (Table 5). In this study, *PKD2* was associated with a reduction of 17 years in median survival to death or onset of ESRD compared with the survival of those without ADPKD. To overcome this case-mix, as well as regional differences, we defined the endpoint for overall survival as death or onset of ESRD. The current true survival figures taking into account survival on dialysis will almost certainly be more optimistic, particularly for those with *PKD1* who have a higher risk of developing ESRD. Although the prevalence of hypertension in affected ADPKD1 individuals increased with age, there was no significant difference between affected males and females ($\chi^2 = 0.14$, $P>0.05$). Against of this finding, males with ADPKD2 had significantly worse overall survival ($P<0.05$), as well as renal survival than females.

DISCUSSION

The diagnosis for presymptomatic ADPKD is mostly done by ultrasonography [19]. The age dependent manifestation of ADPKD renders imaging techniques less effective diagnostic tools than genetic studies. *PKD1* is a big gene that generates a 14-kb transcript, and there are 3 regions highly homologous with the α-globin genes at its 5' end [20]. Mutations falling within the homologous regions are difficult to identify [21]. With the discovery of microsatellite markers closely associated with the *PKD1* and *PKD2* gene loci, PCR amplification of these markers is increasingly being applied on presymptomatic and antenatal diagnosis [22, 23].

In the present study, we used six markers for *PKD1* and *PKD2* genes. Our survey of the allele frequencies of the microsatellites in the local population (Table 1) provided data for a rational choice of markers in future ADPKD genetic linkage studies. KG8 and 16AC2.5 had the highest heterozygosity rates and PIC values and were the most informative markers for the *PKD1* locus. SM7 had intermediate PIC values. *PKD2* markers were not very informative individually, but were highly informative when used in combination. AFM155xe11 had a low PIC value. The results of this study demonstrate a significant locus heterogeneity in ADPKD in Iran. The proportion of families linked in *PKD1* is estimated to be 78%. In recent study, two-point linkage analysis with 16p and 4q allelic markers around the *PKD1* and *PKD2* locus was performed using a LOD method of scoring as implemented in a computer program [24]. The frequency of the *PKD1* was assumed to be 0.001 and *PKD2* 0.0001. A positive LOD score for *PKD1* linkage, in association with the high prior risk, enhances the posterior probability of linkage to *PKD1* locus and a positive LOD score for *PKD2* linkage, in association with the high prior risk, shows probability of linkage to *PKD2* locus.

Table 5. Comparison of ADPKD-1 and -2 in Iranian patients.

	At-risk subjects <30 years old with renal cysts (%)	All subjects <40 years old with hypertension (%)	Subjects with cysts <40 years old with renal insufficiency (%)	Mean age of end-stage renal disease (yr)
ADPKD-1	48	27	11	56
ADPKD-2	11	8	0	73

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In our study, haplotypes were constructed in a number of ADPKD families using respective markers. Assignment of the disease gene loci was performed following phasing and haplotype construction and genotype/phenotype correlations were deduced from the constructed haplotypes. In studying the genotype/phenotype correlations, we have focused on our patient's phenotype and found that high blood pressure is more prevalent in ADPKD patients than in non-affected relatives. We found out that women with *PKD2* had better blood pressure situation and renal survival than men, but this could not seen among *PKD1* patients (Fig. 2). On the influence of sex, only two studies were performed, one of them was in contrast with our findings [2]. That study detected a sex effect for individuals from *PKD1* families as well as other families in which linkage was not informative. Another one confirmed our findings about sex influence in *PKD2* patients [25]. One possible reason for the survival of woman contrast man patients is that, testosterone stimulates fluid secretion and solute transport in Madin-Darby canine kidney cells and may contribute to cyst expansion [26].

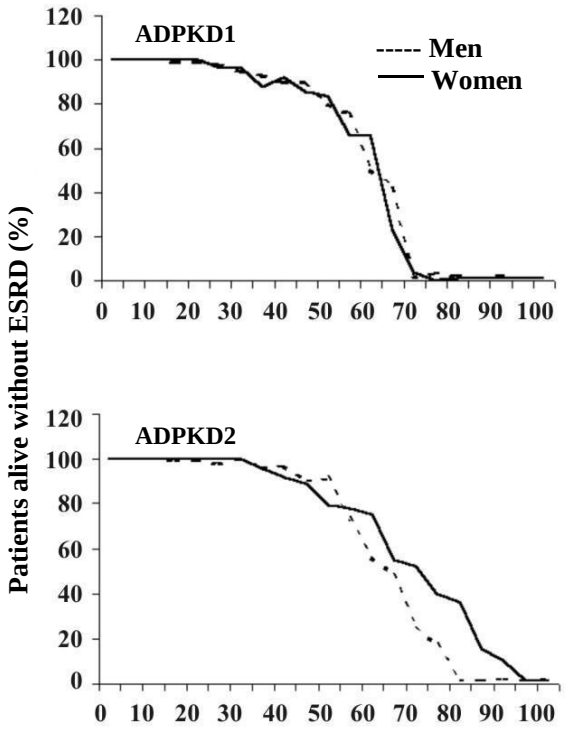

Fig. 2. Cumulative probabilities of survival to end-stage renal disease (ESRD) or death for men and women.

Our study confirming the fact that *PKD2* is a milder form of the disease. Although the age of ESRD seems not to be affected by promptly and effectively treated in order to avoid vascular complications. A clearer appreciation of the relative frequency of complications among people with *PKD1* and *PKD2* has the potential to provide practical management guidance for those affected with ADPKD. Because of the technical demands and expense of both mutation detection and linkage analysis in ADPKD, identification of the genotype is not currently possible in the majority of cases. Nevertheless, the findings of a higher prevalence of hypertension and the poorer cumulative overall survival probability in *PKD1* than in *PKD2* support views that hypertension in ADPKD has a deleterious effect, particularly on renal survival, although the more rapid progression of *PKD1*-associated disease may be due to pathogenetic mechanisms unrelated to hypertension. Although our data have confirmed that *PKD2* is clinically milder than *PKD1*, the adverse effect on survival shows that it cannot be regarded as a benign disorder.

In the course of our study, we encountered some limitations of genetic linkage studies and some of investigated families have no linkage to either of the known gene loci, the absence of clear linkage in these families does not necessarily imply the presence of another disease locus [27] and the existence of a third locus for ADPKD remains uncertain at this study. A number of potential confounders, including genotyping errors, no paternity, misdiagnosis, and bilineal disease, could lead to false exclusion of linkage and will need to be vigorously excluded in these families. But having more siblings within the families, generally gives more information for haplotype construction and data interpretation [11].

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