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Linkage Analysis of Autosomal Dominant Polycystic Kidney Disease in Iranian Families through *PKD1* and *PKD2* DNA Microsatellite Markers

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Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a heterogeneous disorder. Two known loci, including *PKD1* (16p13.3) and *PKD2* (4q21), as well as a third locus that is not clearly identified, cause ADPKD.
Objectives: The aim of this study was to assess the genetic linkage of 4 linked microsatellite markers of PKD genes (*PKD1* and *PKD2*) to ADPKD for genetic screening of familiar PKD patients in Yazd.
Methods: This familial case-control study was conducted among 18 families. The linkage analysis was performed using 2 pairs of polymorphic microsatellite markers that are closely linked to the *PKD1* gene (16AC2.5 and KG8) and 2 other pairs closely linked to the *PKD2* gene (D4S231 and D4S423). These markers were detected through PCR of tandem repeats method and polyacrylamide gel electrophoresis.
Results: The disease was linked to *PKD1* about 77.8%, *PKD2* 16.7% of the families, and to neither gene in 5.5%, according to LOD scores and allele segregation analysis. It also found relatively high heterozygosity and polymorphism information contents (PIC) values for 3 markers including 16AC2.5 (PIC: 0.798) for *PKD1* gene and D4S423 (PIC: 0.807) as well as D4S231 (PIC: 0.741) for *PKD2* gene. However, it seems that KG8 marker has no significant linkage to the *PKD1* gene (PIC: 0.329) among Yazd PKD patients.
Conclusions: These results show similarity to another report from Iranian families and according to these similar results, it seems that 16AC2.5 and D4S423 markers would provide an improved framework for genetic screening of ADPKD patients among familiar PKD patients in Yazd.

Keywords: ADPKD, Linkage, *PKD1*, *PKD2*

1. Background

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic nephropathy characterized by the presence of fluid-filled cysts primarily in the kidneys. This disease is one of the most common hereditary disorders in humans. The incidence of ADPKD is about 1 in 500 to 1 in 1000 in western countries, however, there is no updated and comprehensive data regarding the polymorphic linkage markers pattern among the Iranian population. Up to 50% of patients with ADPKD require renal replacement therapy by 60 years (1). The frequency of ADPKD is high as compared to other prominent genetic disorders, approximately 10, 15, and 20 times higher than sickle cell anemia, cystic fibrosis, and Huntington's disease, respectively. Another form of PKD is autosomal recessive and rare with an incidence of 1 in 20,000 (2).

ADPKD is genetically heterozygous and the mutations occur in the *PKD1* gene located on chromosome 16p13.3 in 85% of the patients (3) and in *PKD2* gene on chromosome 4q21-23 in 15% of the patients (4). The presence of the third gene related to ADPKD in some patients is unclear and does not show linkage to either the *PKD1* or *PKD2* genes (3, 4). The mutations of *PKD1* and *PKD2* genes can produce identical renal and extrarenal manifestations. *PKD2* patients develop the above symptoms at a later age with less intensity as compared to *PKD1* patients (1).

The *PKD1* is a long gene with 46 exons (750 Kbps length), 970 known pathogenic mutations, and there are many homology regions with another part of the genome that make it a hard case for mutation detection. However, *PKD2* has a shorter size, which has fewer problems for direct screening for mutation in compare to *PKD1*. Beyond this, the recently wide progress in sequencing methods has eliminated this problem, however, using a former

method like polymorphic microsatellite linkage analysis is acceptable and cheap. The ADPKD screening and predict pre-symptomatic prenatal ADPKD by use of microsatellite markers closely linked to these loci is commonly used in clinical genetics (5-7).

In this regard, we performed this analysis to assess the genetic linkage of 4 microsatellite markers of polycystic kidney disease genes (*PKD1* & *PKD2*) in ADPKD among 18 Iranian affected families living in Yazd.

2. Methods

2.1. Cases and Controls

The samples were collected from 94 individuals of 18 unrelated ADPKD affected Iranian families (from Yazd) in 2016. This familial case-control study had at least 2 affected and 2 - 3 unaffected members from every family, although most of the families were larger. A total of 47 of the introduced patients by Yazd renal diseases charity foundation, who had a confirmed diagnosis considering their special conditions such as age of the patients (29.04 ± 23.76 years), family history, clinical symptoms, related therapies, and number of cysts in the kidney, were entered into our study (Table 1). The genetic pedigrees were drawn based on a completed questionnaire of each patient. The Ethics Committee of Yazd Reproductive Science Center approved the study protocol and the signed consent after ensure understanding were obtained from all participants (ethic code: IR.SSU.RSI.REC.1395.34).

Table 1. Demographic and Clinical Data

Characteristics	No. (%)
Gender	
Women	19 (40.5)
Men	28 (59.5)
Family History	
Positive	45 (95.8)
Negative	2 (4.2)
Death	
Positive	39 (83)
Negative	8 (17.1)
Clinical symptom	
Cyst in both kidneys	43 (91.5)
Kidney stone	18 (38.3)
Blood pressure	26 (55.3)
Renal pain	29 (61.7)
Blood in the urine	14 (29.8)
Treatment	
Drug treatment	20 (42.5)
Dialysis	10 (21.3)

2.2. Microsatellites Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the phenol-chloroform method. The quality and quantity of the extracted DNA were checked by NanoDrop spectrophotometer (ND-1000) and gel electrophoresis.

Four microsatellite markers on NCBI map viewer and uniSTS data on chromosome 4q and 16p were selected and separated from each other by ~10 cM (KG8, 16AC2.5, D4S423 and D4S231) that the type of these markers is (AC)n repeated (5-8). Using the Ensemble genome database, we checked the correct sequence and distance of the STR marker and disease loci. Two microsatellite markers (16AC2.5 and KG8) are localized within 350kb of the *PKD1* gene (5, 9-11). D4S423 and D4S231 (specific markers) were used to assess linkage to *PKD2* (5, 12, 13).

The primers used for PCR were: KG8 (IF: CTCCCAGGGTGGAGGAAGGTG, IR: GCAG GCACAGCCAGCTCCGAG) (109 - 126 N), 16AC2.5 (IF: AAGGCTGGCAGAGGAG GTG, IR: CAGTTGTGTTTCCTAATCGGCG) (109 - 142 N), D4S423 (IF: TTGAGTAGTTCC TGAAGC AGC, IR: CAAAGTCCTCCATCTTGAGTG (103 - 121 N), D4S231 (IF: ACTATTCAGT GCTAGGAGTTCCC, and IR: GCATCAACTTGGGGAGATCC) (144 - 157 N). Briefly, the target sequence was amplified in 20 μL reaction cocktail containing Mix Red-$Mgcl_2$ (1.5 mM, Amplicon), an equal amount of forward and reverse primer (0. 5 pmol/μL) and genomic DNA (25 ng). Cycle amplification was performed in the Master Cycler (Eppendorf 5331, Germany), (5 minutes at 95°C as an initial denaturation, 35 cycles of 30 seconds at 95°C, 1 minute at an annealing temperature (64°C for KG8, 67°C for D16S291 and D4S423, 58 °C for D4S231), 1 minute at 72°C and 10 minutes at 72°C for final extension). 5 mL of mixture was loaded on a 12% polyacrylamide gel containing 0.5 × TBE buffer for resolving the markers. The gel was run for 5 hours with 100 voltage; after electrophoresis and colored by silver nitrate, the gel was scanned and the allele patterns were analyzed manually. The size of alleles and the informative marker are different in different populations and the sample size and number of evaluated markers are effective in the detection and selection of the informative marker.

2.3. Linkage Analysis and Haplotype Construction

Due to more suitability for a genetic linkage study, the large families were investigated and the evidence of the linkage to *PKD1* and *PKD2* loci was obtained by using of flanking microsatellite polymorphisms. The calculation of LOD scores for this two-point linkage analysis was done via the FASTLINK software. The resultant data in each family were integrated by means of Bayesian weighting formula for the likelihood estimation of a family to one or

other locus (14). The genotyping of individuals includes descendent and married-in patients, where extracted and the founders of genotypes were reconstructed. The relative location of *PKD1* and *PKD2* satellite markers are illustrated in Figure 1. The haplotypes of them in members of ADPKD families were also shown in Figure 2.

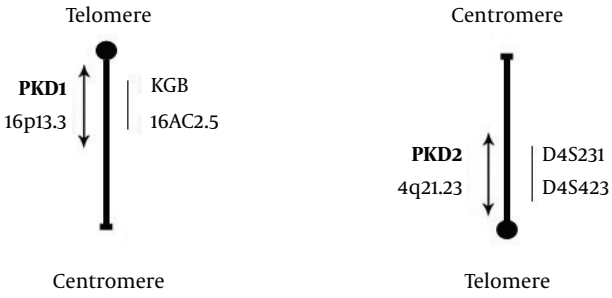

Figure 1. The Relative Locations of the Microsatellite Markers of *PKD1* on Chromosome 16 and *PKD2* on Chromosome 4

3. Results

D4S423, D4S231, and 16AC2.5 show significant linkage with *PKD2* and *PKD1*, respectively.

The heterozygosity of 18 unrelated families was analyzed. Sixteen different sizes of alleles were seen in 4 markers as presented in Tables 2 and 3. According to the findings 16AC2.5, D4S423, and D4S231 markers are suitable for linkage analysis. There were 5 alleles found for 16AC2.5 marker in our understudied population. The HET and PIC values for these markers were also determined 0.782 and 0.798, respectively. Our finding shows D4S423 and D4S231 had both, 4 alleles. Their HET values were 0.840 and 0.775 and their PIC values were 0.807 and 0.741, respectively. Based on our findings, the evaluated microsatellite markers had the acceptable range for HET and PIC values in our linkage analysis on *PKD1* and *PKD2* among the ADPKD patients (Table 2). The LOD scores of our linked microsatellite markers to *PKD1* and *PKD2* were determined. This two-point linkage analysis showed these markers were informative and located at the most flanking locus in each family. The θ values of the maximum LOD scores are also presented in Table 4.

3.1. KG8 Shows Insignificant Linkage with PKD1

The alleles of KG8 marker were 3 forms that the HET and PIC values for this marker were 0.340 and 0.329, respectively. These qualities for HET and PIC were in an acceptable range, however, the calculated LOD and θ values of the maximum LOD scores were not signified regarding KG8 linkage to *PKD1* (Table 4).

Table 2. Number and Size of Alleles, heterozygosity of Polymorphic Markers Linked to *PKD1* and *PKD2* in the Iranian Population

Markers	Number of Alleles	Alleles Size, bp	HET[a]	PIC[b]
PKD1				
KG8	3	109, 118, 126	0.340	0.329
16AC2.5	5	109, 113, 122, 130, 142	0.782	0.798
PKD2				
D4S423	4	103, 107, 117, 121	0.840	0.807
D4S231	4	144, 148, 153, 157	0.775	0.741

[a] Heterozygosity
[b] Polymorphic information content

Table 3. The Frequency of the Marker Alleles Linked to *PKD1* and *PKD2* in the Iranian Population

Genes and Alleles	No. (%)
PKD1	
KG8	
109	27 (57.5)
118	16 (34.1)
126	4 (8.4)
16AC2.5	
109	2 (4.2)
113	3 (6.4)
122	3 (6.4)
130	15 (31.9)
142	24 (51.1)
PKD2	
D4S231	
103	2 (4.2)
107	2 (4.2)
117	14 (29.8)
121	29 (61.8)
D4S423	
144	23 (48.9)
148	11 (23.4)
153	7 (14.9)
157	6 (12.8)

3.2. The Linkage Analysis and Genotype of the ADPKD Families

The LOD scores and allele segregation analysis showed the linkage to *PKD1* was possible approximately 77.8% in 10 families (pedigrees K1-K4, K6, K7, K10-K13, K16, and K17) and this possibility for *PKD2* was 16.7% in 7 families (pedigrees K3, K8, K9, K14, K15 and K18).

LOD scores K5 family were mostly negative and linkage to neither of the 2 PKD loci could be assumed. All families with linkage to *PKD1* and *PKD2* or to either *PKD1* or *PKD2* were selected for screening the further mutation (Table 5).

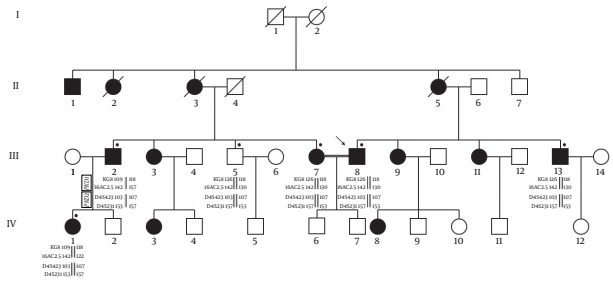

Figure 2. A Pedigree That Show the Polycystic Kidney Disease with Autosomal Dominant Inheritance.

Table 4. Pairwise Z Values for Linkage Between ADPKD and Chromosome 4 Markers

	Markers	LOD Scores						
		θ = 0.0	θ = 0.01	θ = 0.03	θ = 0.05	θ = 0.07	θ = 0.1	θ = 0.15
PKD1	KG8	0.28	0.28	0.27	0.26	0.25	0.23	0.20
	16AC2.5	2.65	2.19	1.51	1.14	0.90	0.76	0.65
PKD2	D4S423	4.25	4.17	3.80	3.52	3.22	2.48	0.98
	D4S231	3.85	3.25	2.90	2.45	2.20	1.35	0.67

Abbreviation: LOD scores, logarithm of the odds; θ, Recombination Frequency.

4. Discussion

The main part of PKD patients has composed of mutation carriers of *PKD1* and *PKD2* genes that these genes are responsible for ADPKD in approximately 85% and 15% of cases, respectively. The percentage of non-autosomal dominant PKD patients is also less than 10% among different populations (3, 4). Several studies from around the world report similar results as mentioned above; for example Mizoguchi et al. in 21 Japanese ADPKD families, including 96 individuals and 57 affected members, reported that 17 families (81%) had linkage to *PKD1*, 2 families (10%); *PKD2* and 2 families did not have linkage to either *PKD1* or *PKD2* (14). Another study was performed by colleagues on 48 Korean families that the results were composed of *PKD1* (79%) and *PKD2* (21%) (15). Moreover, the similar rate of the genetic heterogeneity has been shown in other populations, such as Argentinians (91%) (16), Bulgarians (73%) (17), and Caucasians (81%) (18). Radpour et al. study, the closest one to our investigation, evaluated 15 Iranian families and reported that the proportion of families linked to *PKD1*, *PKD2*, or to other genes was 73%, 13%, and 13%, respectively (5).

Our allele frequencies of *PKD1* and *PKD2* markers (16AC2.5, KG8, D4S423 and D4S231) were not similar to earlier reports in Caucasian ethnics (8, 10, 19-21), however, our results were compliance with Radpour et. al in an Iranian population (5). For instance, among Spanish ADPKD families (48 ADPKD-affected families), it was reported that 7 alleles for D4S231 (HET: 0.71, Zmax: 4.28) as well as 9 for D4S423 (HET: 0.83, Zmax: 9.03) markers for linkage to *PKD2* (8) and 8 different alleles for the KG8 marker and 10 alleles for 16AC2.5 for linkage to *PKD1* (19). There is also another study on 30 Hungarian ADPKD-affected families that reported 12 alleles for D16S663 marker, while 16AC2.5, KG8, D4S1563, and D4S2462 had 10, 8, 12, and 11 alleles, respectively (21).

In summary, according to the results, D4S423 (HET: 0.84, PIC: 0.80), D4S231 (HET: 0.77, PIC: 0.74) and 16AC2.5 (HET: 0.78, PIC: 0.79) had the highest heterozygosity rates as well as PIC values and were the most informative markers for *PKD1* and *PKD2* loci to diagnose ADPKD while the less informative marker was KG8 (HET: 0.34, PIC: 0.32) for *PKD1* locus in the population. Therefore, 1 marker linked to the *PKD1* gene (16AC2.5) and the 2 markers linked to *PKD2* genes (D4S423 and D4S231) were informative for screening of ADPKD patients in our population.

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Footnote

Conflict of Interest: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors and no conflict of interest is declared.

Table 5. Linkage Analysis and Genotype in 18 Iranian ADPKD Families

Families	*PKD1*		*PKD2*		
	Zmax	θmax	Zmax	θmax	Genotypes
K1	0.402	0.00	-1.21	0.099	*PKD1*
K2	0.450	0.00	-4.00	0.005	*PKD1*
K3	-0.942	0.00	1.42	0.005	*PKD2*
K4	0.308	0.00	-1.48	0.019	*PKD1*
K5	∞-	0.00	-1.45	0.015	-
K6	0.720	0.014	-2.45	0.013	*PKD1*
K7	-0.608	0.00	-1.43	0.015	*PKD1*
K8	-0.905	0.00	0.112	0.125	*PKD2*
K9	-0.306	0.00	3.89	0.006	*PKD2*
K10	0.698	0.00	-3.00	0.113	*PKD1*
K11	0.567	0.014	-2.05	0.007	*PKD1*
K12	1.20	0.00	-5.80	0.004	*PKD1*
K13	0.725	0.00	-2.60	0.015	*PKD1*
K14	-0.603	0.00	0.115	0.013	*PKD2*
K15	-0.305	0.00	3.20	0.019	*PKD2*
K16	0.502	0.014	-3.00	0.006	*PKD1*
K17	-0.935	0.00	-3.15	0.014	*PKD1*
K18	-1.30	0.00	4.00	0.088	*PKD2*

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