

# Heterozygosity analysis of polycystic kidney disease 1 gene microsatellite markers for linkage analysis of autosomal dominant polycystic kidney disease type 1 in the Iranian population

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**Background:** Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic cause of end-stage renal disease. Although imaging techniques are a means of accurate diagnosis when the cysts appear in the third or fourth decades of the patient's life, they are of little value for early diagnosis. Genetic tests are required for preimplantation genetic diagnosis, decision-making for kidney donation to an affected relative. Although mutation of the polycystic kidney disease (*PKD1*) gene is solely responsible for the most cases of ADPKD, direct genetic testing is limited by the large size of this gene and the presence of many mutations without hot spots. Therefore, indirect diagnosis with linkage analysis using informative microsatellite markers has been suggested. **Materials and Methods:** In this study, we assessed the informativeness of the *PKD1* gene markers D16S475, D16S291, and D16S3252 in Iranian population. Using specific primers, fluorescent polymerase chain reaction (PCR) was performed on genomic DNA extracted from fifty unrelated individuals. PCR products were analyzed by the ALFexpress DNA sequencer system, and the number and frequency of alleles were determined to calculate the heterozygosity (HET) and polymorphism information content (PIC) values. **Results:** We found that the HET and PIC values for the D16S475 marker are 0.92 and 0.91, respectively. These two values are 0.82 and 0.80 for D16S291 and 0.50 and 0.47 for D16S3252, respectively. **Conclusion:** Based on this data, D16S475 and D16S291 are highly and D16S3252 is moderately informative for indirect genetic diagnosis of *PKD1* mutations in this population.

**Key words:** Autosomal dominant polycystic kidney disease, linkage analysis, microsatellite markers, polycystic kidney disease 1

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## INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a common hereditary kidney disease, with a frequency of 1 in 400–1 in 1000 in the general population.<sup>[1]</sup> This progressive disorder is caused by polycystic kidney disease (*PKD*)-1 mutation in 85% of the cases and *PKD*-2 mutation in most of the remaining ADPKD patients.<sup>[2,3]</sup> ADPKD is caused by growing cysts in the kidney tubules often leads to renal failure and end-stage renal disease.<sup>[4]</sup> Besides the impairment of kidney function, ADPKD

can also affect other organs such as the liver, pancreas, and spleen through the occurrence of growing cysts.<sup>[5]</sup> This multisystemic disorder is associated with cerebral and aortic aneurysms which cause sudden death in 8% of the patients.<sup>[6]</sup> Typically, ADPKD is diagnosed with the detection of cysts in the kidneys by imaging techniques. However, as the disease is late onset, these techniques are not suitable for diagnosis in children and young adults. Genetic tests allow the early detection of the disease which is important for genetic counseling, preimplantation genetic diagnosis, kidney donation from a young relative, or selection of individuals for

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the screening of aneurysms.<sup>[7,8]</sup> However, direct mutation detection of this disorder are not straightforward due to the large size of the genes, extensive missense mutations dispersed almost all over the genes, and the existence of *PKD1* homolog parts in the genomes.<sup>[9,10]</sup> Therefore, indirect genetic testing based on linkage analysis can be considered for early detection of ADPKD.<sup>[11]</sup> This method is highly dependent on the presence of informative markers. Marker informativeness is population dependent and the appropriateness of markers should be assessed in local populations.<sup>[12]</sup> The *PKD1* gene region contains different polymorphic markers. Among the microsatellite markers reported in the *PKD1* gene region, two extragenic markers, i.e., D16S475 and D16S291, and one intragenic marker, i.e., D16S3252 have been shown to have a high degree of heterozygosity (HET).<sup>[13-16]</sup> However, investigation of these markers has been rarely performed in the Iranian population. In this study, the HET and polymorphism information content (PIC) values for the *PKD1* markers D16S475, D16S291, and D16S3252 were determined in a normal population of Iran.

## MATERIALS AND METHODS

### Sample preparation and DNA extraction

Peripheral blood samples were obtained from fifty unrelated, healthy individuals in Isfahan, Iran. DNA was extracted using the genomic DNA isolation kit (Genet Bio, Seoul, South Korea) according to the instructions of the manufacturer.

### Primer design

The sequences of microsatellite markers were obtained from the ensemble database.<sup>[17]</sup> Primers were designed with PrimerPlex 2 V2.5 software (PREMIER Biosoft, Palo Alto CA, USA) for multiplex polymerase chain reaction (PCR) [Table 1]. Primer synthesis and labeling were performed by Macrogen (Seoul, South Korea).

### Microsatellite genotyping

Using fluorescent PCR, DNA samples were genotyped for D16S475, D16S291, and D16S3252 with specific

primers. PCR reactions were carried out in total volume of 25  $\mu$ l containing 1 mM MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase, 2.5  $\mu$ l 10X PCR buffer, 0.2 mM dNTP mix (all from CinnaGen, Tehran, Iran), 2  $\mu$ l DMSO (85%; MP Biomedicals, Santa Ana, California), 2  $\mu$ l glycerol (20% v/v; Merck, Darmstadt, Germany), 5  $\mu$ M reverse primer, 5  $\mu$ M Cy5-labeled forward primer, and 50 ng genomic DNA. Reaction conditions were as follows: 94°C (5 min), 10 cycles of 94°C (30 s), 68°C (30 s), and 72°C (30 s), followed by 30 cycles of 94°C (30 s), 60°C (30 s), and 72°C (30 s), and then a final extension step at 72°C for 5 min. After an initial examination with 2% agarose gel, the Cy5-labeled PCR products were studied using the automated laser fluorescence ALFexpress system (Amersham, Pharmacia Biotech, Roosendaal, Netherlands) for fragment size analysis.

### Data analysis

PowerMarker V3.25 software was applied to perform the subsequent statistical analyses, including allele frequency, observed and expected HET values, PIC value, and testing the Hardy–Weinberg equilibrium (HWE).<sup>[18]</sup>

## RESULTS

To determine the informative markers for ADPKD1 genetic testing, genotyping of the markers linked to the *PKD1* gene, D16S475, D16S291, and D16S3252, was performed for fifty unrelated healthy individuals by fluorescent PCR followed by fragment analysis. The genomic location of the markers is shown in Figure 1a and a representative graph of fragment analysis for each marker is shown in Figure 1b. Genotyping analysis of D16S475, D16S291, and D16S3252 revealed the presence of 19, 9, and 6 alleles in the studied Iranian population, respectively. Allele frequencies of the D16S475 and D16S291 markers have approximately uniform distribution, a key feature of informative markers [Figure 2]. The most common alleles for the D16S475 and D16S291 markers have a frequency of 0.14 and 0.27, respectively. However, for the D16S3252 marker, one allele alone had a frequency of 0.69 that significantly differs from the frequency of other alleles, implying a relatively lower HET for this marker.

To assess the informativeness of the markers, observed HET, expected HET, and PIC values were calculated based on allele frequencies [Table 2]. D16S475 and D16S291 with HET and PIC values >0.8 are highly informative markers. In addition, the D16S3252 marker with lower HET and PIC values is moderately informative in this population. The calculated HWE *P* value for D16S3252 was above 0.05. However, for D16S475 and D16S291, the population is not assumed to be in the HWE (*P* < 0.05).

**Table 1: Characteristics of selected microsatellite markers including repeated sequence markers, sequence of primers, and polymerase chain reaction products size**

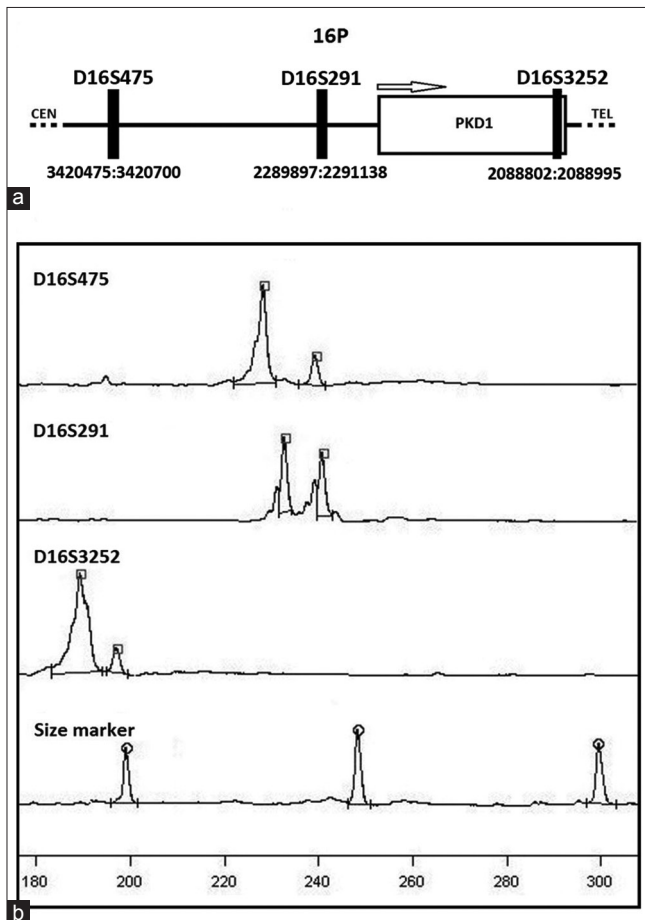
Marker	Marker type	Primer sequence (5'→3')	Product size (bp)
D16S475	(GA) <sub>n</sub> A(AAGA) <sub>n</sub>	TGAACTGAGGTCTACCACTG AGAACTACTGGCAGGAACAGA	207-243
D16S291	(CA) <sub>n</sub>	GATTACAGGCATGAACCACCA AGCAAGAAGCAGGCAGACA	233-249
D16S3252	(CA) <sub>n</sub>	GTACACAGAAGCAGGCACAG GGCAAGTAGCAGGACTAGGC	183-199

## DISCUSSION

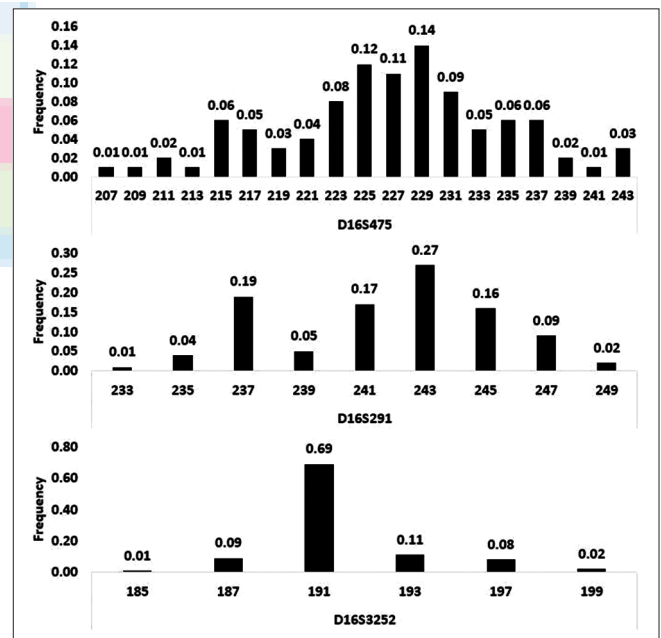
Indirect genetic testing by linkage analysis is a reliable and practical approach for ADPKD diagnosis in clinical settings. This method is highly reliant on the presence of informative genetic markers. However, the informativeness of markers, expressed by HET and PIC values, is population dependent and should be assessed in the society that the test is planning to be established. In this study, the HET of three microsatellite markers for the *PKD1* gene, as the most responsible gene for ADPKD, was determined in Iranian population.

In the present study, microsatellite genotyping was performed for unrelated healthy individuals. We showed that D16S291 is a highly informative marker. This finding is consistent with another study on the Iranian population by Radpour *et al.*<sup>[19]</sup> We demonstrated that the D16S3252 marker is moderately informative, whereas it was highly informative in that study. This discrepancy may be due to the diversity of the Iranian population and difference of the subpopulations of these two studies. Furthermore, these two markers are known as suitable markers for ADPKD genetic testing in most studied populations.<sup>[8,13-15]</sup> In addition, for the first time, we found D16S475 to be highly informative in our population. This marker has also been reported as a suitable marker in some other studies.<sup>[16]</sup>

In this study, fifty healthy individuals were randomly chosen. The selection of this sample size was based on a previous study by Hale *et al.* that showed only 25–30 individuals per population is enough to accurately estimate the number and frequency of common alleles and the calculation of related parameters.<sup>[20]</sup> Determining the frequency and HET of rare alleles are of little value for



**Figure 1:** In this study, we assessed the informativeness of the polycystic kidney disease 1 gene markers D16S475, D16S291, and D16S3252 in an Iranian population. The genomic position of the polycystic kidney disease 1 gene microsatellite markers is shown (a). A representative graph of fragment analysis for each marker is demonstrated (b)



**Figure 2:** The number and frequencies of alleles for three microsatellite markers D16S475, D16S291, and D16S3252 for the polycystic kidney disease 1 gene were determined

**Table 2: Number of genotypes, number of alleles, observed and expected heterozygosity, PIC value, and Fisher’s exact P value of three microsatellite markers for linkage analysis of ADPKD1 in the Iranian population**

Marker	Number of genotypes	Number of alleles	Heterozygosity		PIC value	Exact (P)
			Observed	Expected		
D16S475	41	19	0.76	0.92	0.91	0.01
D16S291	20	9	0.54	0.82	0.80	0.00
D16S3252	10	6	0.44	0.50	0.47	0.44

PIC = Polymorphism information content, Exact (P)= Fisher’s exact P value: The probability of significant deviation from Hardy–Weinberg equilibrium

practical applications. A drawback of this study was the inability to show that the population is in the HWE for the D16S475 and D16S291 markers. This could be due to the relatively small sample size and the high number of alleles for these two markers. In spite of this limitation, the reported HET parameters for these two markers can be considered as acceptable estimations for clinical purposes.

## CONCLUSION

In this study as a first step of indirect genetic testing for ADPKD, the HET of three microsatellite markers of the *PKD1* gene was determined. However, the suitability of more markers for the *PKD1* gene and the other responsible gene, *PKD2*, remains to be investigated in future studies.

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## Conflicts of interest

There are no conflicts of interest.

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