Integrative Biosciences 10: 65-71, 2006

Integrative
Biosciences
http://www.ibiosci.or.kr

Characterization of Microsatellite Markers Closely Linked with *PKD* Loci in the Korean Population

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Abstract: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is one of the most common inherited renal disorders in the world. Mutations in *PKD1* located on chromosome 16p13.3 are responsible for 85% of all the ADPKD patients whereas mutations in *PKD2* on chromosome 4q21-23 are responsible for the rest of the cases. Genetic heterogeneity and the problems of mutation detection in *PKD1* suggest that linkage analysis is an important approach to study the genetics of ADPKD. To evaluate the availability of six (CA)n microsatellite markers for the linkage analysis of ADPKD in the Korean population, we examined the allele frequencies and heterozygosities of the markers. With the exception of KG8, five markers were highly informative, with PIC values over 0.5, but the PIC value of KG8 marker was less informative than other five markers because of the low number of alleles. Therefore, this study will be useful in linkage analysis for ADPKD families in the Korean population.

Key words: Autosomal dominant polycystic kidney disease (ADPKD), PKD1 gene, PKD2 gene, linkage analysis, Koreans

ADPKD (Autosomal Dominant Polycystic Kidney Disease, ADPKD) is a genetically heterogeneous disorder and can be caused by a mutation in at least three different genes. In 1985, Reeders et al. reported linkage of PKD to D16S85 (3'HVR), a highly polymorphic locus on the short arm of chromosome 16, located close to the α-globin gene cluster. Since then, a number of additional polymorphisms in closer proximity to this locus, now designated as the *PKD1* locus, have been identified (Breuning et al., 1990; Wolff et al., 1988; Germino et al., 1990, 1992; Hyland et al., 1990; Somlo et al., 1992) and used for refined mapping of the *PKD1* gene. The gene has recently been identified and characterized (European Polycystic Kidney Disease Consortium, 1994). It lies within a complex duplicated genomic region and produces a large transcript of ~14-kb. Because of its size and complexity of the gene, it can be expected to cause significant problems in mutation detection.

Population studies have demonstrated that, in a small proportion of families, the disease is not linked to the chromosome 16 markers (Kimberling et al., 1988; Romeo et al., 1988; Norby et al., 1989), thus suggesting the existence of a second locus, designated *PKD2*. In Europe, PKD that is not linked to the chromosome 16 locus has been estimated to account for about 14% of affected families (Peters and Sandkuijl, 1992). It has been suggested that mutation in *PKD2* result in a milder clinical phenotype (Parfrey et al., 1990; Ravine et al., 1992; Bear et al., 1992; Gabow et al., 1992). In 1996, *PKD2* gene was cloned and sequenced (Mochizuki et al., 1996). It essentially accounts for all non-PKD1 patients, although a third locus, apparently very rare, has also been implicated and is still of unknown chromosomal location (Daoust et al., 1995; de Almeida et al., 1995).

Genetic heterogeneity and the problems of mutation detection in *PKD1* suggest that linkage analysis is an important approach to study the genetics of ADPKD. Although the microsatellite markers closely linked in PKD loci have been well characterized to perform the genetic analysis in Caucasians, the usefulness of these markers for linkage analysis in the Korean populations is unknown. To provide these markers for diagnosis of ADPKD, we analyzed the genetic variations of the six (CA)n markers in the Korean population.

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MATERIALS AND METHODS

Subjects

A total of one hundred twelve individuals including 70 affected ADPKD patients were analyzed in this study. The affected individuals were ascertained through Department of Internal Medicine, Kangbuk Samsung Hospital. The diagnosis of ADPKD was based on the presence of at least two cysts in one kidney or one cyst in each kidney in an at-risk person under 30 year of age, the presence of at least two cysts in each kidney in an at-risk person between 30 and 59 year of age, and at least four cysts in each kidney for those persons at-risk aged 60 year and older (Ravine et al., 1994). Informed consent was obtained for all participants.

Isolation of genomic DNAs

Genomic DNAs were extracted from blood using the method described by Lahiri and Nurnberger (1991).

Whole blood was collected in a vacutainer tube containing 100 μl of 15% EDTA. 5 ml of blood was transferred into a 15 ml cornical tube and 5 ml of low salt buffer (TKM1) containing 10 mM Tris-HCl pH7.6, 10 mM $MgCl_2$ and 2 mM EDTA was added. To lyse the cells, 125 μl of Nonidet P-40 was added, mixed by inversion several times, and centrifuged at 1,000 × g for 10 min at room temperature. The supernatant was poured off and the nuclear pellet was saved. The pellet was washed in 5 ml of TKM1 buffer and centrifuged as before. The pellet was gently resuspended in 0.8 ml of high salt buffer (TKM2) containing 10 mM Tris-HCl pH7.6, 10 mM KCl, 10 mM $MgCl_2$, 0.4 M NaCl and 2 mM EDTA and 50 μl of 10% SDS was added. And then the whole suspension was mixed thoroughly by pipetting back and forth several times and incubated for 10 min at 55°C. After incubation, 300 μl of 6 M NaCl was added in the tube and centrifuged at 12,000 rpm for 5 min in microcentrifuge. The supernatant was saved and 2 volumes of absolute ethanol were added. The tube was inverted several times until DNA was precipitated. The precipitated DNA was collected and put into a microcentrifuge tube containing 1 ml of 70% ethanol for overnight. The ethanol was discarded and dried the pellet in a speed-vac. The dried DNA was resuspended in TE buffer (0.5 ml of 10 mM Tris-HCl, 1 mM EDTA pH8.0), and stored in the refrigerator until used.

DNA analysis

The samples were typed using three (CA)n microsatellites for *PKD1* (SM7-D16S283, 16AC2.5-D16S291, and KG8-*PKD1*) on chromosome 16p13.3 and three (CA)n microsatellites for *PKD2* on chromosome 4q21-23 (D4S1534, D4S2460, and D4S423). KG8 is an intragenic marker, at the 3' end of the *PKD1* gene, whereas the other microsatellites are proximal to *PKD1*. D4S1534 is proximal to the *PKD2* gene and D4S2460 and D4S423 are distal.

The PCR was performed essentially according to the method of Saiki et al. (1989). Approximately 100 ng of genomic DNA was amplified in a total volume of 25 μl containing 10 pmol of each primer; 200 μM each of dGTP, dATP, and dTTP; 25 μM dCTP; 1.5 mM $MgCl_2$; 10 mM Tris-HCl pH 8.3; 50 mM KCl; 0.01% gelatin; 1 μCi ^{32}P-dCTP; and 1 unit of *Taq* DNA polymerase. Three different sets of amplification conditions were used. For the primer set of KG8, denaturation was carried out at 94°C for 30 sec, and annealing at 58°C for 30 sec, and extension at 72°C for 30 sec. For the primer set of 16AC2.5, denaturation was carried out at 94°C for 1 min, and annealing at 65°C for 1 min, and extension at 72°C for 30 sec. Amplification conditions for primer sets SM7, D4S1534, D4S2460, D4S423 were denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 30 sec. A total of 30 amplification cycles were performed in a Perkin Elmer 480 thermal cycler.

Aliquots of PCR products were mixed with one-half volume of formamide dye solution (98% formamide, 10 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol), heated to 95°C for 5 min, and electrophoresed on a 6% denaturing polyacrylamide sequencing gel. Following electrophoresis, each gel was fixed for 30 min in a bath of 15% methanol and 5% acetic acid. Autoradiography of the dried gel with an intensifying screen was performed at −80°C for 12-72 hr. Alleles were sized by comparison with an M13 mp18 sequencing standard electrophoresed on the same gel.

Table 1. Heterozygosity and PIC values for microsatellite markers used in this study

Marker	No. of chromosomes	No. of alleles	Heterozygosity (%)	PIC	No. of chromosomes	Published number of alleles	Published heterozygosity (%)	Published PIC	References
SM7	218	9	0.6606	0.6320	200	11	0.6480	0.6264	Harris et al, 1991
16AC2.5	218	11	0.7415	0.7110	259	9	0.8068	0.7790	Peral et al, 1994
KG8	224	5	0.4056	0.3369	189	8	0.5789	0.5539	Peral et al, 1994
D4S1534	212	10	0.7998	0.7743	56	6	0.7700	0.7351	Weinssenbach et al, 1992
D4S2460	210	5	0.5775	0.5342	28	6	0.7300	0.7011	Weinssenbach et al, 1992
D4S423	214	11	0.7234	0.6834	56	9	0.8200	0.8092	Weinssenbach et al, 1992

Table 2. Allele frequencies of 3 markers on chromosome 16 used in this study

Allele	Allele length (bp)	Allele frequencies				χ^2	df	*P*
		Korean		Caucasian				
SM7		*n* = 218		*n* = 200		65.114	11	0.0001
A1	107	-	-	1	0.0050			
A2	103	2	0.0092	-	-			
A3	101	4	0.0184	3	0.0150			
A4	99	15	0.0688	12	0.0600			
A5	97	36	0.1651	9	0.0450			
A6	95	9	0.0413	11	0.0550			
A7	93	12	0.0550	8	0.0400			
A8	91	118	0.5413	113	0.5650			
A9	89	1	0.0046	28	0.1400			
A10	87	21	0.0963	5	0.0250			
A11	85	-	-	9	0.0450			
A12	81	-	-	1	0.0050			
16AC2.5		*n* = 218		*n* = 259		58.641	11	0.0001
B1	129	2	0.0092	-	-			
B2	125	2	0.0092	-	-			
B3	123	1	0.0046	-	-			
B4	121	1	0.0046	2	0.0077			
B5	119	31	0.1422	22	0.0849			
B6	117	41	0.1881	58	0.2239			
B7	115	20	0.0917	58	0.2239			
B8	113	6	0.0275	37	0.1429			
B9	111	19	0.0871	11	0.0425			
B10	109	94	0.4312	65	0.2509			
B11	107	1	0.0046	5	0.0193			
B12	105	-	-	1	0.0039			
KG8		*n* = 224		*n* = 189		235.58	7	0.0001
C1	130	-	-	8	0.0423			
C2	128	2	0.0089	24	0.1270			
C3	126	-	-	2	0.0106			
C4	124	1	0.0045	6	0.0317			
C5	122	57	0.2544	118	0.6243			
C6	120	163	0.7277	2	0.0106			
C7	118	1	0.0045	12	0.0635			
C8	116	-	-	17	0.0900			

Statistical analysis

Allele frequencies of the six (CA)n markers were calculated directly by dividing the number of positive for allele by the number of chromosomes tested. Heterozygosities and PIC (polymorphism information content) values were calculated according to the method of Bostein et al. (1980). Chi-square independence test was used to test the significance of differences in allele frequencies between Koreans and Caucasians or between Controls and ADPKD. The statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

We characterized the number of alleles, their sizes, heterozygosity, and PIC of the markers described above, in a group of 105 to 112 unrelated individuals (Table 1). The allele frequency distributions between Korean and Caucasian population were significantly different (Table 2). For SM7, the second most frequent allele was drastically different, A5 (97-bp) in Koreans and A9 (89-bp) in Caucasians ($p < 0.05$). Also, A10 (87-bp), the third frequent allele in our

Table 3. Allele frequencies of 3 markers on chromosome 4 used in this study

Allele	Allele length (bp)	Allele frequencies				χ^2	df	*P*
		Korean		Caucasian				
D4S1534		*n* = 212		*n* = 56		18.606	9	0.0288
D1	162	1	0.0047	-	-			
D2	160	9	0.0425	-	-			
D3	158	27	0.1273	5	0.0893			
D4	156	68	0.3207	14	0.2500			
D5	154	22	0.1038	6	0.1071			
D6	152	10	0.0472	10	0.1786			
D7	150	7	0.0330	-	-			
D8	148	15	0.0708	2	0.0357			
D9	146	52	0.2453	19	0.3393			
D10	140	1	0.0047	-	-			
D4S2460		*n* = 210		*n* = 28		25.696	5	0.0001
F1	191	2	0.0095	1	0.0370			
F2	189	14	0.0667	7	0.2590			
F3	187	127	0.6048	8	0.2960			
F4	185	28	0.1333	2	0.0560			
F5	183	39	0.1857	9	0.3330			
F6	179	-	-	1	0.0190			
D4S423		*n* = 214		*n* = 56		38.796	13	0.0002
G1	125	-	-	1	0.0179			
G2	123	-	-	2	0.0357			
G3	121	-	-	1	0.0179			
G4	119	20	0.0935	9	0.1607			
G5	117	8	0.0374	6	0.1071			
G6	115	87	0.4065	16	0.2857			
G7	113	11	0.0514	5	0.0893			
G8	111	66	0.3084	9	0.1607			
G9	109	10	0.0467	-	-			
G10	107	1	0.0047	-	-			
G11	105	1	0.0047	-	-			
G12	103	7	0.0327	7	0.1250			
G13	101	2	0.0093	-	-			
G14	97	1	0.0047	-	-			

population, showed a lower frequency in Caucasians ($p < 0.05$). In contrast, A11 (85-bp), the fifth frequent allele in Caucasians was not observed in Koreans ($p < 0.05$). Several allele frequencies of 16AC2.5 exhibited significant differences between populations. The B10 allele (109-bp) was the most frequent in both populations, its frequency in Koreans was higher ($p < 0.05$). Allele frequencies of B5 (119-bp) and B9 (111-bp) were higher than that of Caucasians, while B7 (115-bp) and B8 (119-bp) decreased in our population. Three new alleles (B1, B2, and B3), which were not found in Caucasians, were observed in the Korean population. The distribution of allele frequency at KG8 in both populations showed unimodal, but the most common allele appears to be different, C6 (120-bp) in Koreans, and C5 (122-bp) in Caucasians. Among six markers included in this study, the heterozygosity and PIC value of KG8 showed the lowest value. This means that KG8 have little information as a genetic maker for ADPKD in our population, although the marker is located in 3'UTR of the *PKD1* gene. While the allele frequencies of *PKD1*-linked markers showed significant differences between Korean and Caucasian populations, those of *PKD2*-linked markers showed milder

Table 4. Comparison of allele frequencies for 3 markers on chromosome 16 between ADPKD patients and controls

Allele	Allele length (bp)	Allele frequencies				χ^2	df	*P*
		Control		ADPKD				
SM7		*n* = 84		*n* = 134		13.391	8	0.0991
A2	103	2	0.0238	-	-			
A3	101	3	0.0357	1	0.0075			
A4	99	2	0.0238	13	0.0970			
A5	97	15	0.1786	21	0.1567			
A6	95	1	0.0119	8	0.0597			
A7	93	5	0.0595	7	0.0522			
A8	91	47	0.5595	71	0.5299			
A9	89	-	-	1	0.0075			
A10	87	9	0.1072	12	0.0895			
16AC2.5		*n* = 82		*n* = 136		7.772	10	0.6511
B1	129	1	0.0122	1	0.0074			
B2	125	-	-	2	0.0147			
B3	123	-	-	1	0.0074			
B4	121	1	0.0122	-	-			
B5	119	14	0.1707	17	0.1250			
B6	117	12	0.1463	29	0.2132			
B7	115	10	0.1220	10	0.0735			
B8	113	2	0.0244	4	0.0294			
B9	111	8	0.0976	11	0.0808			
B10	109	34	0.4146	60	0.4412			
B11	107	-	-	1	0.0074			
KG8		*n* = 86		*n* = 138		7.588	4	0.1079
C2	128	1	0.0116	1	0.0073			
C4	124	1	0.0116	-	-			
C5	122	28	0.3257	29	0.2101			
C6	120	55	0.6395	108	0.7826			
C7	118	1	0.0116	-	-			

variations (Table 3). For D4S1534, allele frequency of D6 (152-bp), the third frequent allele in Caucasians is higher than that of Koreans ($p < 0.05$). The frequency distribution of two alleles (F2, F3) at D4S2460 showed a significant difference in both populations ($p < 0.05$). Several allele frequencies of D4S423 also exhibited significant differences. Allele frequency of G8 (111-bp) in Koreans was higher than that of Caucasians ($p < 0.05$). With the exception of KG8, five markers were highly informative, with PIC values over 0.5, but the PIC value of KG8 marker was less informative than other five markers because of the low number of alleles. All the microsatellites except D4S1534 marker did not display a statistically significant difference in allele distribution between ADPKD and controls (Table 4 and 5). D8 (148 bp) allele of D4S1534 may have the association with the phenotype, but the further study with large size of samples will be required for it.

The choice of genetic markers for human linkage analysis is based on both the location of the marker with respect to a putative disease and the degree of polymorphism of the marker. The latter may vary significantly between populations resulting in a different PIC, which may affect the choice of an appropriate marker for a linkage study. Therefore, information about the marker in the targeted population is useful in design of experiment. Furthermore, in some methods of linkage analysis, accurate calculations of likelihood are dependent upon correctly specifying the allele frequencies of the markers that are used (Risch, 1990).

Table 5. Comparison of allele frequencies of 3 markers on chromosome 4 between ADPKD patients and controls

Allele	Allele length (bp)	Allele frequencies				χ^2	df	*P*
		Control		ADPKD				
D4S1534		*n* = 82		*n* = 130		17.882	9	0.0366
D1	162	-	-	1	0.0077			
D2	160	6	0.0732	3	0.0231			
D3	158	7	0.0854	20	0.1538			
D4	156	24	0.2927	44	0.3385			
D5	154	8	0.0976	14	0.1077			
D6	152	3	0.0366	7	0.0538			
D7	150	2	0.0244	5	0.0385			
D8	148	12	0.1463	3	0.0231			
D9	146	20	0.2439	32	0.2461			
D10	140	-	-	1	0.0077			
D4S2460		*n* = 84		*n* = 126		5.395	4	0.2491
F1	191	-	-	2	0.0159			
F2	189	4	0.0476	10	0.0794			
F3	187	58	0.6905	69	0.5476			
F4	185	10	0.1190	18	0.1428			
F5	183	12	0.1429	27	0.2142			
D4S423		*n* = 84		*n* = 130		6.284	10	0.7908
G4	119	7	0.0833	13	0.1000			
G5	117	2	0.0238	6	0.0462			
G6	115	35	0.4167	52	0.4000			
G7	113	4	0.0476	7	0.0538			
G8	111	29	0.3452	37	0.2846			
G9	109	4	0.0476	6	0.0462			
G10	107	1	0.0119	-	-			
G11	105	-	-	1	0.0077			
G12	103	1	0.0119	6	0.0462			
G13	101	1	0.0119	1	0.0077			
G14	97	-	-	1	0.0077			

Acknowledgment

This research was supported by Kyungpook National University Redearch Fund, 2005.

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[Received March 10, 2006; accepted May 4, 2006]