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Genotypes of autosomal dominant polycystic kidney disease in Japanese

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Abstract Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary disorders. The prevalence of the ADPKD genotype in the Caucasian and Latin populations has been reported. Here, we used linkage analysis to demonstrate the prevalence of the genotype and the correlation between phenotypes and genotypes among 21 Japanese ADPKD families consisting of 96 individuals and including 57 affected members. Six polymorphic markers, each linked to either the polycystic kidney disease 1 (*PKD1*) or polycystic kidney disease 2 (*PKD2*) gene, were used for polymerase chain reaction analysis. Seventeen families (81%) showed linkage to *PKD1*, two families (10%) showed linkage to *PKD2*, and two families did not show linkage to either *PKD1* or *PKD2*. One of the *PKD1*-linked families was indicated to have different mutations of *PKD1* gene in the same family. *PKD2*-linked families did not have milder symptoms than *PKD1*-linked families.

Key words ADPKD · Genotype · Linkage study · Polymorphism · PKD1 · PKD2

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary disorders. ADPKD is usually adult onset (MacDermot et al. 1998; Watnick et al. 1999). The prevalence of ADPKD in the Japanese population has been estimated as 1/4033 (Higashihara et al. 1998), which is much less than among Caucasians. Previous linkage studies showed that approximately 80%–85% of ADPKD families are positively linked to the polycystic kidney disease 1 (*PKD1*) gene and most of the remainder are linked to the polycystic kidney disease 2 (*PKD2*) gene (Wright et al. 1993; Bogdanova et al. 1995; Iglesias et al. 1997). Only a few families not linked either to *PKD1* or *PKD2* have been reported in Caucasian and Latin populations (Daoust et al. 1995; Almeida et al. 1995, 1999; Turco et al. 1996; Ariza et al. 1997). Correlations between genotypes and phenotypes of ADPKD have also been investigated in those populations (Hateboer et al. 1999). Many researchers have suggested that the severity of symptoms is greater in patients with *PKD1* than in those with *PKD2*, but the relationship is still not clear. We performed linkage analysis to demonstrate the distribution of the genotypes and the difference of phenotypes among the genotypes in 21 Japanese ADPKD families.

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Subjects and methods

Patient recruitment. ADPKD families were recruited nationwide through their family doctors at the request of the ADPKD research group, supported by the Ministry of Health and Welfare of Japan. Family members were evaluated ultrasonographically with symptomatic affected subjects showing at least five more cysts bilaterally in the kidneys. Family doctors took blood samples from individuals after obtaining informed consent. Each family in this study included at least two affected members and a minimum of two unaffected siblings, parents, and/or children.

DNA preparation. Genomic DNA was extracted from peripheral blood lymphocytes by standard methods, using phenol/chloroform extraction and isopropanol precipitation.

Polymerase chain reaction and data analysis. To assess linkage to PKD1, two intragenic (KG8, I42) and four other

markers for *PKD1* were used. I42 is a polymorphic marker within intron 42 of the *PKD1* gene. This marker has sequences of tandem repeats, for example, between nucleotides 50155 and 50436. KG8 is a microsatellite marker in the 3′ untranslated region of the *PKD1* gene (Snarey et al. 1994). Microsatellite markers 16AC2.5, CW2, SM7 are localized within 350 kb of the *PKD1* gene (Harris et al. 1991; Thompson et al. 1992; Viribay et al. 1994). Microsatellite marker D16S521 is located distal to the *PKD1* gene (Coto et al. 1995). To assess linkage to *PKD2*, six markers, D4S231, D4S1534, D4S1542, D4S1563, D4S1544, and D4S414 (Mills et al. 1992), were used. The 5′ end of one of the paired primers was labeled with X-rhodamine isothiocyanate (XRITC) and used for polymerase chain reaction (PCR). PCR was performed as reported previously with slight modifications. PCR products were separated electrophoretically on a 40-cm length of 8% polyacrylamide gel containing 7 M urea at 18 W for 4 h. Allele patterns were analyzed with an FMBIO 100 fluoro-imager (Takara, Kusatsu, Japan).

Results

Heterozygosity of *PKD1* and *PKD2* markers

Twelve markers used in this study were selected as polymorphic markers. The heterozygosity in the Japanese population was analyzed using genomic DNA from 80 unrelated Japanese individuals. In I42 marker there were five different sizes of alleles and 47% of the heterozygosities was shown (Table 1). All were indicated to be useful markers for linkage analysis.

Linkage analysis

Haplotype analyses were carried out in 21 Japanese families using six markers linked to *PKD1* and six to *PKD2*. The families consisted of 96 individuals, 45 males and 51 females, including 57 affected members. Three-point linkage analysis was performed between the distal and proximal markers for *PKD1* and *PKD2* that were informative and located at the most flanking locus in each family. Maximum lod scores are shown for each respective θ value (Table 2). We determined the genotype by the positive or negative lod scores to the *PKD1* or *PKD2* markers. Sixteen families showed linkage to *PKD1*. Two families, K38 and K84, showed linkage to *PKD2*, and three families, K68, K71 and K123, did not show linkage to either *PKD1* or *PKD2*. In family K68, which did not show linkage to either *PKD1* or *PKD2*, only two out of three affected sibs had the same haplotype. The nonsense mutation Q4124X in exon 45 was found only in the two members with the same haplotype, but not in the one with the different haplotype (Mizoguchi et al. 2001). This family was also screened for *PKD2* gene mutations, but no mutation was found. Because of the mutation data, family K68 was included in the group of *PKD1*-linked families.

Clinical symptoms and genotypes

Comparative clinical symptoms of 57 affected individuals with *PKD1*, *PKD2*, and non-*PKD1* or *PKD2* genotypes are shown in Table 3. Forty-nine percent of these individuals were on treatment for hypertension, 39% were positive for liver cysts, and 23% were on hemodialysis; 4% had ischemic heart disease, and 5% had cerebral hemorrhage. In the *PKD1*-linked families, 4 out of 20 patients had hypertension in their second decade, and 67% of the patients had hemodialysis treatment before 60 years of age.

Table 1. Heterozygosity of polymorphic markers linked to *PKD1* and *PKD2* in the Japanese population

Marker	Product size (bp)	Number of alleles	Heterozygosity
PKD1			
D16S521	239–265	7	0.54
KG8	145–149	3	0.46
I42	233–310	5	0.47
16AC2.5	108–134	13	0.88
CW2	104–128	11	0.91
SM7	84–102	11	0.75
PKD2			
D4S231	157–169	4	0.47
D4S1534	136–164	14	0.56
D4S1542	210–224	4	0.36
D4S1563	176–200	4	0.79
D4S1544	112–120	3	0.45
D4S414	222–236	5	0.63

PKD, polycystic kidney disease

Table 2. Linkage analysis and genotype in 21 Japanese ADPKD families

	PKD1		*PKD2*		
Family	Z	θ max	Z	θ max	Genotype
K5	0.301	0	−1.11	0.0196	PKD1
K6	0.602	0	−4.00	0.0050	PKD1
K22	0.602	0	−4.00	0.0050	PKD1
K38	−0.947	0	1.490	0.0050	PKD2
K48	0.301	0.0178	−2.48	0.0196	PKD1
K56	0.426	0	−3.57	0.0990	PKD1
K60	0.301	0	−1.41	0.0990	PKD1
K68	−2.71	0.0178	−3.14	0.0148	non-PKD1, PKD2[a]
K71	−∞	0	−1.41	0.0990	non-PKD1, PKD2
K74	1.200	0	−2.44	0.0148	PKD1
K79	0.602	0	−1.41	0.0990	PKD1
K80	1.200	0	−3.70	0.0050	PKD1
K84	−∞	0	0.113	0.0990	PKD2
K96	0.727	0	−2.50	0.0196	PKD1
K97	0.903	0	−6.09	0.0148	PKD1
K103	0.903	0.0178	−6.80	0.0990	PKD1
K111	0.301	0	−1.41	0.0990	PKD1
K113	0.902	0.0178	−4.00	0.0050	PKD1
K123	−0.0512	0	−3.10	0.0990	non-PKD1, PKD2
K127	0.726	0.0178	−3.70	0.0050	PKD1
K131	0.602	0	−3.04	0.0148	PKD1

ADPKD, autosomal dominant polycystic kidney disease; Z, lod score
[a]Two out of three affected members in this family had a nonsense mutation of the *PKD1* gene

Table 3. Distribution of clinical symptoms and genotypes among 57 affected individuals

	PKD1	*PKD2*	non-*PKD1*, *PKD2*	Total
Number affected	47 (100%)	5 (100%)	5 (100%)	57 (100%)
Hypertension	20 (43%)	4 (80%)	4 (80%)	28 (49%)
Liver cyst	18 (38%)	3 (60%)	1 (20%)	22 (39%)
Hemodialysis	9 (19%)	2 (40%)	2 (40%)	13 (23%)
Ischemic heart disease	1 (2%)	1 (20%)	0	2 (4%)
Cerebral hemorrhage	2 (4%)	1 (20%)	0	3 (5%)

Discussion

Genotypes in the Japanese population

The family size in our study was relatively small (average 4.5 individuals). Sixteen of the ADPKD families were positively linked to *PKD1*. Since a *PKD1* mutation was found in family K68, this family was included among those with the *PKD1* genotype, so there were 17 *PKD1*-linked families (81%) among the 21 families. This is almost same proportion of genetic heterogeneity reported previously in other populations, such as among Caucasians (81%) (Wright et al. 1993), Bulgarians (73%) (Bogdanova et al. 1993), and Argentinians (91%) (Iglesias et al. 1997). There has been some doubt about the existence of a third *ADPKD* gene (Paterson and Pei 1998). Recently, bilineal disease and trans-heterozygotes were found in a large ADPKD family that had been previously assigned to a third PKD-gene linked family. *PKD1* and *PKD2* mutations segregated independently in this large family (Pei et al. 2001). Family K68 showed the possibility of bilineal ADPKD disease because of different mutations of the *PKD1* gene, although only one mutation was identified (Mizoguchi et al. 2001). In family K123, with no linkages to either *PKD1* or *PKD2*, one 11-year-old child was affected, but another, 13 years old, was not affected at the time of the study. This child may acquire cysts with age. In the two families (K71 and K123) not linked to either gene, no mutations in the unique region of *PKD1* or in exons of *PKD2* have yet been identified. Thus, we could not determine whether a third gene for ADPKD is present in these families.

Clinical symptoms and genotypes

The occurrence of clinical symptoms was lower than reported previously, but patients with hypertension and undergoing hemodialysis treatment showed early onset (Gabow 1993). Eight of the patients in this study were younger than 20 years old, and none had any clinical symptoms at the time of their diagnosis. More individuals may become symptomatic in the future. Differences in the severity of symptoms between patients with PKD1 and those with PKD2 were not clear in this study because only two families were linked to *PKD2*. But these two *PKD2*-linked families did not display milder symptoms than the average among *PKD*-linked families.

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