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Association of the *ACE* Gene Polymorphism with the Progression of Autosomal Dominant Polycystic Kidney Disease

Renin-angiotensin system is considered important in the genesis of hypertension and development of end-stage renal disease (ESRD) in autosomal dominant polycystic kidney disease (ADPKD). The angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism has been associated with susceptibility to the development of some renal diseases. We investigated the association of ACE gene polymorphism with the progression to hypertension and ESRD in 108 patients with ADPKD. The ACE I/D polymorphism was amplified with the flanking primers by polymerase chain reaction. In patients genotyped for ACE gene polymorphism, the frequencies of DD (15%), ID (51%) and II (34%) genotypes were similar to those of the general population. Of the 108 patients, 64 (59%) developed hypertension and 24 (22%) reached ESRD at the time of study. The prevalence of hypertension was not significantly different among the three genotypes. The mean renal survival time was 53±6 yr in II genotype, 55±10 yr in ID genotype and 52±9 yr in DD genotype which was not significantly different among them. Cumulative renal survival was not significantly different either. There was no association of ACE gene polymorphism with the prevalence of hypertension and renal survival in ADPKD. We suggest that ACE I/D polymorphism is not an important modifying gene in the progression of ADPKD.

Key Words: *Kidney, Polycystic, Autosomal Dominant; Peptidyl Dipeptidase A; Polymorphism (Genetics); Kidney Failure; Hypertension*

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disorder (1) that is characterized by the development of hypertension, end stage renal disease (ESRD) and sometimes intracranial aneurysm (ICA) rupture (2, 3). Hypertension is a frequent trait of patients with ADPKD, due to the activation of renin-angiotension system (RAS) by the ischemia secondary to cyst compression (4). Approximately 50% of the patients reach ESRD by 60 yr of age (5). One of the devastating manifestations is ruptured ICA, and the reported frequency of ICA is about 5% (3).

Patients with ADPKD exhibit marked non-uniformity in the progression of the disease. The age at onset of renal failure ranges from 2 to 80 yr (2). It is suggested that the principal factor determining the progression is the genetic type of the disease, since the median renal survival is 53 yr in PKD1 (gene on 16p) versus 68 yr in PKD2 (gene on 4q) (6). However, intrafamily heterogeneity has been shown in disease expression (7), and discrepancies between twins also have been reported (8). Some environmental and genetic factors have been proposed to influence the progression of ADPKD. Modifier genes have been identified in mice (9) and it may explain the discrepancy between genotype and phenotype.

Activation of RAS contributes to the early development of hypertension of ADPKD (4). Increased amount of tubular renin is found in the ADPKD kidney, and angiotensin II is an important growth factor in the cystic epithelium (10). RAS plays an important role in the pathogenesis of ADPKD. We hypothesized that the angiotensin converting enzyme (ACE) I/D polymorphism is a determinant of the clinical course in ADPKD. In this study, we investigated the impact of the *ACE* genotype on clinical features such as hypertension and ESRD.

SUBJECTS AND METHODS

We included 108 patients from 88 families with ADPKD at Kangbuk Samsung Hospital and 9 related nephrology centers. The diagnosis was made based on their family history, medical history and abdominal ultrasound. Ultrasonographic criteria for diagnosis included two cysts either unilateral or bilateral in an at-risk person less than 30 yr of age, two cysts in each kidney in an at-risk person age 30 to 59 yr, and at least four cysts in each kidney in individuals over age 60 (11). Blood pressure was measured with a standard mercury sphygmomanometer on the arm, and hypertension was defined as diastolic blood pressure greater than 90 mmHg or taking antihypertensive medication. The renal survival time was defined as the age of long-term renal replacement therapy was started.

Genomic DNA was isolated from peripheral blood lymphocytes by the salting out procedure (12). *ACE* gene I/D polymorphism was detected by performing PCR as described by Rigat et al. (13). A set of primers was used to encompass the polymorphic region in intron 16 of the *ACE* gene. The PCR contained 400 ng of genomic DNA, 20 pmol of each primer, 200 mM of dNTPs, 2 units of *Taq* DNA polymerase, and 1.5 mM $MgCl_2$. DNA was amplified for 30 cycles (denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min). The PCR product was visualized after electrophoresis in 2% agarose gels. The PCR product was a 190-bp fragment in the absence of the insertion and a 490-bp fragment in the presence of the insertion. To prevent mistyping of ID types, we used a specific primer for the insertion (14) whenever a DD genotype was found. In case of an I-allele, a fragment of 408-bp was present, while in case of a true DD homozygote, no such band was present. Electrophoresis of PCR products in each *ACE* genotypes are shown in Fig. 1.

Statistical analysis was performed using the one-way ANOVA to evaluate the phenotypic variations among the II, ID, and DD genotypes. A chi-square test was used to compare the qualitative variables. Kaplan-Meier survival curves were used to present the time until the onset of ESRD or censoring. Cox proportional hazard models were used to compare the hazard of ESRD between the different polymorphism groups. Statistical analysis was performed using SPSS software (SPSS Advanced Statistics™ 7.0 Uptodate, Chicago, 1996). A value of $p<0.05$ was considered statistically significant.

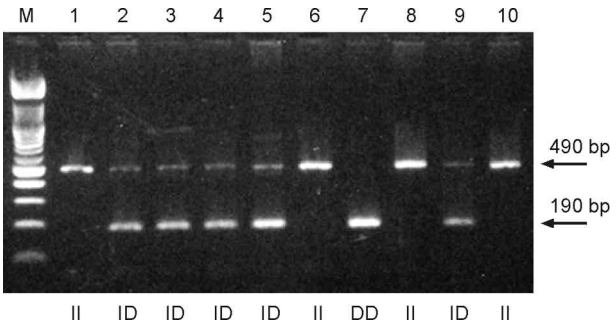

Fig. 1. Examples of 3 patterns of the *ACE* insertion/deletion polymorphism. II homozygotes have a band of 490 bp, DD homozygotes have a band of 190 bp and ID heterozygotes have both bands. Lane 1-5, normal control; 6-10, ADPKD patients.

RESULTS

We studied 108 subjects with ADPKD (58 men, 50 women), with a mean age of 46±14 yr (Fig. 2). Baseline characteristics of the 108 ADPKD patients are illustrated in Table 1. Of them, 64 (59%) were hypertensive and 24 (22%) reached ESRD at a mean age of 54±9 yr (range 36-69 yr). Among the 108 patients, the frequencies of *ACE* genotypes II, ID and DD were 37 (34%), 55 (51%) and 16 (15%), respectively (Table 1). The genotype distribution was similar to that in the general population as previously reported in Korea. The prevalence of hypertension was not significantly different among the three genotypes (χ^2=1.057, df=2, p=0.589). In those patients with ESRD, the genotype frequencies were not significantly different among the three genotypes (χ^2=0.369, df=2, p=0.832). The incidence of ruptured ICA was greater in DD type than in other types.

We calculated the cumulative renal survival time for each genotype. The mean renal survival time was: II, 53±6 yr; ID, 55±10 yr; DD, 52±9 yr. These differences in the age of reaching ESRD were not statistically signifi-

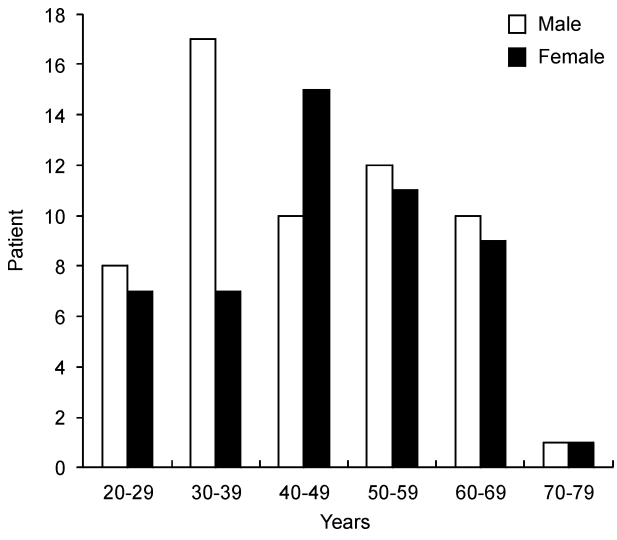

Fig. 2. Age and sex distribution in 108 ADPKD patients.

Table 1. Distribution of *ACE* I/D polymorphism and clinical characteristics in patients with ADPKD

	ACE genotype				
	II	ID	DD	Total	p-value
No. of patients	37 (34%)	55 (51%)	16 (15%)	108	
Age (yr)	46±13	46±14	45±13	46±14	
Sex (men:women)	18:19	28:27	12:4	58:50	
Hypertension	24 (65%)	30 (55%)	10 (63%)	64 (59%)	*p=0.589
ESRD	7 (19%)	13 (24%)	4 (25%)	24 (22%)	*p=0.832
Age of ESRD (yr)	53±6	55±10	52±9	54±9	*p=0.785
Ruptured ICA	1	0	3	4	

Values expressed as mean±SD
ESRD, end stage renal failure; ICA, intracranial aneurysm
*No statistically significant differences versus genenotype distribution

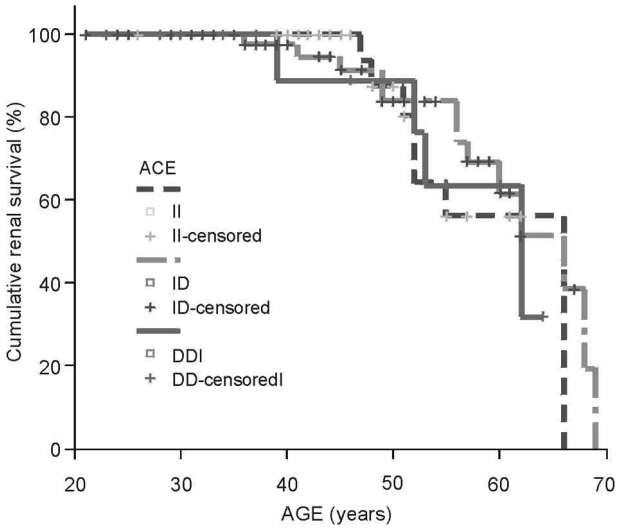

Fig. 3. Cumulative renal survival for each *ACE* gene polymorphism.

cant (p=0.785). Cumulative renal survival (Fig. 3) was also not different among the three genotypes.

DISCUSSION

Patients with ADPKD exhibit marked non-uniformity in the progression. The principal factor determining the progression is the genetic type of the disease. However, intrafamily heterogeneity has been shown in disease expression (7) and discrepancies between the twins have also been reported (8). In accordance with previous studies, the *ACE* gene deletion allele accelerated the progression of renal disease (15, 16), but others have failed to support this (17). Thus, the subject still remains controversial. We investigated the impact of the *ACE* genotype on clinical features such as hypertension and ESRD in patients with ADPKD.

In this study, the frequencies of II, ID and DD genotypes in ADPKD patients were not different from those in non-ADPKD Korean population (18). The I and D allele frequencies in Korea were different compared to the *ACE* allele frequencies in the white population, but similar with those of Chinese or Japanese subjects (19-21). The frequencies of I allele was higher than that of D allele in Korean population, but the frequencies of D allele was higher than that of I allele in the white population (22, 23).

Hypertension is potentially a treatable factor associated with a faster progression to ESRD or ruptured ICA. Hypertension is the most frequent, and is affecting approximately 60 percent of adults before the onset of renal failure, and more than 80 percent of patients with ESRD (2, 3). The activation of RAS is considered to derive by compression of the renal arteries by enlarged cysts. We could not find any association between *ACE* gene polymorphism and hypertension. In this study, there were 4 cases of ruptured ICA. The incidence of ruptured ICA was greater in DD type than in other types, but we could not treat this data statistically due to the small number of events. ESRD is the most serious renal complication. Approximately, 50 percent of the patients will reach ESRD by the age of 60 yr (2, 5, 6), but the age of onset of ESRD is variable. In this study, the genotype frequencies were not significantly different among the three genotypes. Cumulative renal survival was also not different among the three genotypes. There was no association of *ACE* gene I/D polymorphism with the prevalence of hypertension and renal survival in ADPKD. Our results suggest that the *ACE* gene I/D polymorphism has no significant effect on the progression of ADPKD.

There are several studies reporting the relationship between the *ACE* gene polymorphism and ADPKD. Uemasu et al. (21) suggested the irrelevance of the *ACE* gene polymorphism in clinical manifestation of 47 patients with ADPKD (including mean age of ESRD and

slopes of 1/serum creatinine). Baboolal et al. (22) studied 189 patients with the PKD1 disease and found that DD genotype conferred a worse renal survival compared with the II and ID genotype. Perez-Oller et al. (23) evaluated 155 patients with the PKD1 disease and reported that DD genotype implied a worse renal prognosis based on both the lower median renal survival time and greater percentage of patients who reached ESRD before the age of 50 yr. Our studies were similar to Uemasu's study, but different to Baboolal's and Perez-Oller's studies. We could not select the PKD1 family in this study, because all the families involved in the study were not analyzed by linkage studies. However, our results confirmed that the *ACE* gene I/D polymorphism had no effect on the progression of ADPKD. At least, the association of ACE gene polymorphism with the progression of ADPKD showed a small degree of significance. This also suggest that *ACE* gene polymophism is less important in the oriental population than in the white population.

In conclusion, we observed that *ACE* gene I/D polymorphism was not implicated in patients with ADPKD nor was it significant in the association of *ACE* gene polymorphism with the progression of ADPKD. These findings need to be confirmed in more studies that involve various ethnical groups with ADPKD. We suggest that *ACE* I/D polymorphism is not an important modifying gene that can explain the clinical variability in the progression of ADPKD. Additional studies on the genetic or environmental factors will be needed to determine why progression of ADPKD is heterogenous in most patients.

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