Angiotensinogen and Angiotensin II Type 1 Receptor Gene Polymorphism in Patients with Autosomal Dominant Polycystic Kidney Disease: Effect on Hypertension and ESRD

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Autosomal dominant polycystic kidney disease (ADPKD), a common genetic disease, is characterized by the development of hypertension and end stage renal disease. An increase in the activity of the renin-angiotensin system, due to a renal ischemia caused by cyst expansion, contributes to the development of hypertension and renal failure in ADPKD. Recently, the angiotensinogen (AGT) gene, M235T, and angiotensin II type 1 receptor (ATR) gene, A1166C, polymorphisms have been associated with the susceptibility to develop hypertension and renal disease. We hypothesized that the AGT M235T and ATR A1166C polymorphisms could account for some of the variability in the progression of ADPKD. Genotyping was performed in 108 adult patients with ADPKD, and 105 normotensive healthy controls, using PCR and restriction digestion. We analyzed the effects of the AGT M235T and ATR A1166C polymorphisms on hypertension and age at the end stage renal disease (ESRD). Of the 108 patients with ADPKD, 64 (59%) had hypertension and 24 (22%) reached the ESRD. The prevalence of hypertension were; [MM+MT], [TT] genotypes, 60%, 59% (p=1.00); [AA], [AC+CC] genotypes, 60%, 50% respectively (p=0.54). The ages at the onset of ESRD were; [MM+MT], [TT] genotypes, 50 ± 9 years, 56 ± 8 years (p=0.07); [AA], [AC+CC] genotypes, 54 ± 8 years, 52 ± 14 years, respectively (p=0.07). There were no differences in the prevalence of hypertension and the ages at the ESRD in relation to the AGT M235T and ATR A1166C polymorphisms. We suggest that the AGT and ATR gene polymorphisms would not have an effect on hypertension or the ESRD in ADPKD.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD), one of the most common genetic disorders, affects 1 in 1,000 persons.[1] The clinical manifestations are the presence of renal cysts, the progressive enlargement of cysts, which can lead to hypertension and end stage renal failure (ESRD). Hypertension is the most frequent complication, with an occurrence in 60 percent of adults before the onset of renal insufficiency,[2] and is a major factor associated with a more aggressive course.[3] Approximately 50 percent of the patients reach ESRD by the age of 60, but the age at onset of renal failure ranges from 2 to 80 years.[2,3] Patents with ADPKD exhibit marked non-uniformity in the progression of the disease. Intrafamilial heterogeneity has been shown in the expression of the disease,[4] and discrepancies between twins have also been reported.[5] It is suggested that some environmental and genetic factors have an influence on the progression of ADPKD. Modifier genes have been identified in mice,[6] and might explain the discrepancies between the genotype and phenotype.

Activation of the renin-angiotensin system (RAS) contributes to the early development of hypertension in ADPKD.[7] Increased amounts of tubular renin are found in the ADPKD kidney,

and angiotensin II is an important growth factor in the cystic epithelium.[8] The RAS plays an important role in the pathogenesis of ADPKD. Recently, molecular genetics studies have linked the gene polymorphism of the RAS gene to primary hypertension, diabetic nephropathy and other renal diseases.[9-11] A point mutation of a nucleic acid, at codon 235 in the angiotensinogen (AGT) gene, leads to an amino acid substitution of threonine for methionine. This AGT M235T polymorphism has been associated with the variation in the plasma concentration of AGT,[12] hypertension and renal disease.[9,10,12] A nucleic acid substitution of adenine (A) with cytosine (C) at the 1166 position in the angiotensin II type 1 receptor (ATR) gene, A1166C, has been identified and associated with hypertension.[11,13] We hypothesized that the AGT M235T and ATR A1166C polymorphisms are determinants of the clinical course in ADPKD. In this study, the impact of the AGT and ATR genotypes, on clinical features, such as hypertension and ESRD, were investigated.

MATERIALS AND METHODS

Patients

The study subjects included 108 patients, from 88 families with ADPKD, at the Kangbuk Samsung Hospital and 9 related nephrology centers, and 105 normotensive controls, collected at the Kangbuk Samsung Hospital during annual medical visits for preventive medicine. The diagnosis of ADPKD was made on the basis of their family history, medical history and abdominal ultrasound. Ultrasonographic criteria for the diagnosis included; two cysts, either unilateral or bilateral, in an at-risk person of less than 30 years of age, two cysts in each kidney of an at-risk person aged between 30 and 59, or at least four cysts in each kidney in an individual over 60.[14] The blood pressure was measured with a standard mercury sphygmomanometer on the arm, and hypertension was defined as a diastolic pressure greater than 90 mmHg, or the taking antihypertensive medication. The renal survival time was defined as the age at which the long-term renal replace-

ment therapy was started.

Methods

Genomic DNA was isolated from peripheral lymphocytes using the salting out procedure.[15] The AGT gene M235T polymorphism was detected by performing PCR, as described by Russ et al.[16] The PCR reaction was carried out in a final reaction volume 30 μL with 15 pM of each primer (5'-CAGGGTGCTGTCCACACTGGACCCC-3' and 5'-CCGTTTGTGCAGGGCCTGGCTCTCT-3'), 200 μM of each dNDT, 1.5 mM MgCl$_2$, 50 mM KCl, 10 mM tris-HCl (pH 8.3), 2 unit Taq polymerase and 200 ng of genomic DNA. The DNA was amplified with 30 cycles (denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min). Then Tth 111 I 1 U was added to the amplified products, and digested for at least 2 hours. The digestion products were visualized, after electorphoresis in 3% agarose gel and ethidium bromide staining. The PCR products were a 165 bp fragment from the M 235 allele and a 141 bp fragment from the T235 allele (Fig. 1). To determine the ATR gene A1166C polymorphism, a simple PCR formatted RFLP assay was used, as described by Hingorani AD et al.[17] The reaction was carried out in a final reaction volume 30 μL with 15 pM of each primer (5'-ATAATGTAAGCT CATCCACCAAGAAG-3' and 5'-TCTCCTTCAAT TC TGAAAAGTACTTAA-3'), 200 μM of each dNDT, 1.5 mM MgCl$_2$, 50 mM KCl, 10 mM tris-HCl (pH 8.3), 2 unit Taq polymerase and 200 ng of genomic DNA. DNA was amplified for 30 cycles (denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, extension at 72℃ for 1 min). Than, Afl II 1 U was added to the amplified products, and digested for at least 2 hours. The digestion products were visualized, after electorphoresis in 3% agarose gel and ethidium bromide staining. The PCR products were a 166 bp fragment inform the A1166 allele and a 139 bp fragment inform the C1166 allele (Fig. 2).

Statistical analysis

All data are reported as the means ± standard deviation (SD). A statistical analysis was performed using the one-way ANOVA and indepen-
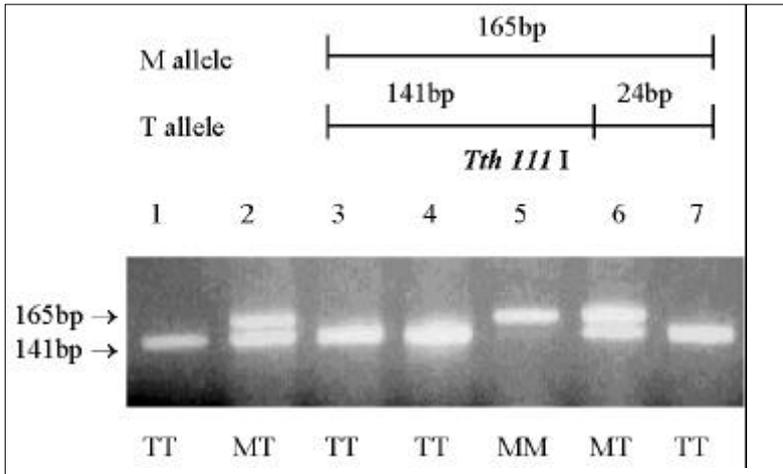
Fig. 1. The M235T polymorphism of the angiotensinogen (AGT) gene. Lanes 1-3 show the normal controls and lanes 4-7 show the patients with ADPKD.
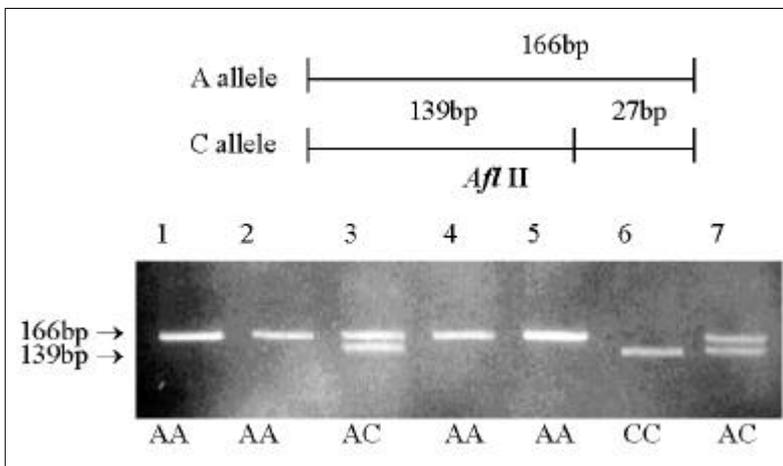

Fig. 2. The A1166C polymorphism of the angiotensin II type 1receptor (ATR) gene. Lanes 1-3 show the normal controls and lanes 4-7 show the patients with ADPKD.

dent sample t-test to evaluate the phenotypic variations of each genotype. A chi-square test was used to compare the qualitative variables, and Kaplan-Meier survival curves to present the time until the onset of the ESRD. Cox proportional hazard models were used to compare the hazard of ESRD between the different polymorphism groups. The statistical analyses were performed using SPSS software (SPSS Advanced Statistics™ version 7.0 Up-to-date, Chicago, 1996). A value of $p < 0.05$ was considered statistically significant.

RESULTS

We studied 108 ADPKD subjects (58 men, 50 women), with a mean age of 46 ± 14 years, and 105 normotensive controls (56 men, 49 women), with a mean age of 46 ± 13 years. Of the 108 patients with ADPKD, 64 (59%) had hypertension and 24 (22%) had reached ESRD at the time of the study. Six patients had cerebrovascular accidents, of which 4 had cerebral aneurysm ruptures. The prevalence of the different genetic polymorphisms of the AGT and ATR genes, in the patients with ADPKD, was as follow: MM genotype 4 (4%), MT genotype 33 (30%), TT genotype 71 (66%), for the AGT gene; and AA genotype 96 (89%), AC genotype 11 (10%), CC genotype 1 (1%), for the ATR gene. All genotypes distributions followed the Hardy-Weinberg equilibrium. The overall frequencies of the M and T alleles were 19% and 81% of the AGT gene, and 94% and 6% for the A and C alleles of the ATR gene, respectively. The allele frequencies of the AGT M235T and the ATR

A1166C polymorphisms showed no statistical difference between patients and the controls (Table 1).

There was no significant difference in the prevalence of hypertension between the [MM + MT] and [TT] genotypes (Table 2), between [AA] genotype and [AC + CC] genotypes (Table 3). The cumulative renal survival times for each genotype were calculated and were as follow: [MM + MT] genotype 50 ± 9 years, [TT] genotype 56 ± 8 years, for the AGT gene ($p > 0.05$); and [AA] genotype 54 ± 8 years, [AC + CC] genotype 52 ± 14 years, for the ATR gene ($p > 0.05$). There was also no difference between the cummulative renal survival between the genotypes ($p > 0.05$) (Fig. 3).

DISCUSSION

The clinical course of ADPKD is highly variable. This variation can be attributed to the genetic heterogeneity: the mutations in the PKD1 gene (gene on 16p) are more severe than those in the PKD2 gene (gene on 4q).[18] Nevertheless, the clinical variability cannot be explained fully by these two different genes. Considerable intrafamilial variability in either the PKD1 or PKD2 families is frequently observed. Environmental, and other genetic modifying factors, can be responsible for this intrafamilial variability.

The RAS has been strongly implicated in the pathogenesis of essential hypertension, cardiovascular disease and progressive renal disease.

Table 1. Genotype and Allele Frequencies for the M235T Polymorphism of the AGT Gene and the A1166C Polymorphism of the ATR Gene in the Controls and the ADPKD Patients

	AGT genotype			Alleles	
	MM	MT	TT	M	T
Control (105)	3(3%)	37(35%)	65(62%)	43(20%)	167(80%)
ADPKD (108)	4(4%)	33(30%)	71(66%)	41(19%)	167(81%)
	$\chi^2=0.594$, df=2, $p=0.743$*				

	ATR genotype			Alleles	
	AA	AC	CC	A	C
Control (105)	94(90%)	11(10%)	0	199(95%)	11(5%)
ADPKD (108)	96(89%)	11(10%)	1(1%)	203(94%)	13(6%)
	$\chi^2=0.979$, df=2, $p=0.613$*				

*Both groups were in Hardy-Weinberg equilibrium.

Table 2. Distribution of the AGT Gene M235T Polymorphism, and the Clinical Characteristics in the 108 ADPKD Patients

	AGT genotype			p-value
	MM + MT	TT	Total	
No. of patients	37(34%)	71(66%)	108	
Mean age (yrs.)	45 ± 12	46 ± 14	46 ± 13	
Sex (M : F)	20 : 17	38 : 33	58 : 50	
Hypertension	22(60%)	42(59%)	64(59%)	*$p=1.0$
ESRD	9(24%)	15(21%)	24(22%)	*$p=0.81$
Age of ESRD (yrs)	50 ± 9	56 ± 8	54 ± 9	*$p=0.07$

Values expressed as mean ± S.D.
*No statistically significant differences versus genotype distribution.

Table 3. Distribution of the ATR Gene A1166C Polymorphism, and the Clinical Characteristics in 108 ADPKD Patients

	ATR genotype			*p*-value
	AA	AC+CC	Total	
No. of patients	96(89%)	12(11%)	108	
Mean age (yrs.)	46 ± 13	45 ± 14	46 ± 13	
Sex (M : F)	50 : 46	8 : 4	58 : 50	
Hypertension	58(60%)	6(50%)	64(59%)	*p=0.54
ESRD	20(21%)	4(33%)	24(22%)	*p=0.46
Age of ESRD (yrs)	54 ± 8	52 ± 14	54 ± 9	*p=0.72

Values expressed as mean ± S.D.
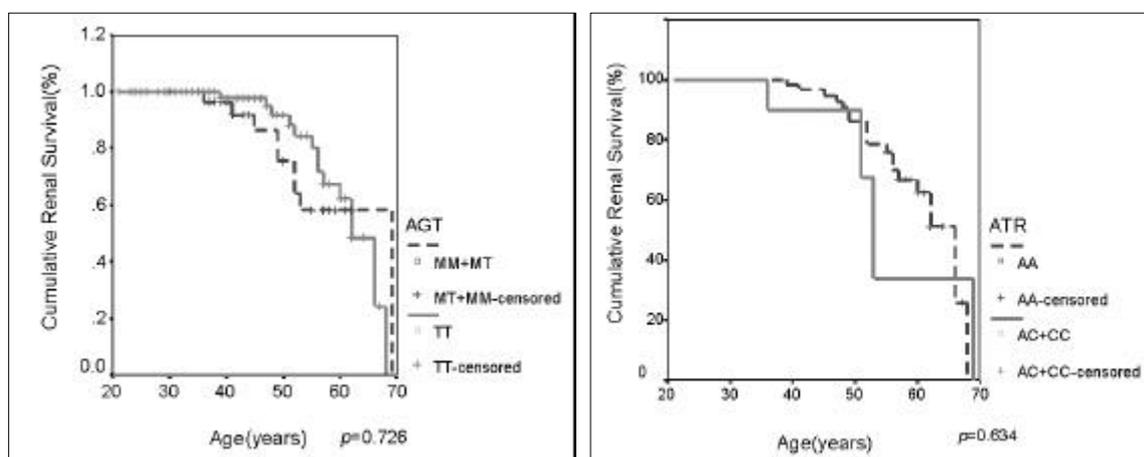*No statistically significant differences versus genotype distribution.

Fig. 3. Cumulative renal survival for each AGT gene M235T and each ATR gene A1166C polymorphisms. There was no difference in age at onset of ESRD between the M235T and A1166C genotypes.

Angiotensin II, as a final product of RAS activity, is both a powerful vasoconstictor, and a potent mediator, of the cellular proliferation and extra-cellular matrix protein synthesis and accumula-tion. These effects contribute to progressive fibro-tic disease in various organ systems. Most of the physiologic effects of the angiotensin II are medi-ated by the angiotensin II type 1 receptor (ATR). Many studies have attempted to relate the genetic variants of RAS to hypertension, and cardio-vascular and renal diseases.[9-11] Kunz, et al.[19] per-formed a meta-analysis to examine the associa-tion between the AGT T235 allele and essential hypertension. They reviewed 11 studies, and 5,493 patients, and confirmed a statistically significant, even though weak, association between the AGT T235 variant and essential hypertension in Cauca-

sians. Staessen, et al.[20] attempted to estimate the association between the AGT T235 polymorphism and various cardiovascular-renal disorders using meta-analysis. They included 69 reports, with an overall sample size of 27,906 subjects, and con-cluded the AGT T235 allele was not associated with atherosclerotic or microvascular complica-tions, but behaves as a marker for hypertension in Caucasians.

The activation of RAS in ADPKD is considered to be derived from the compression of the renal arteries by enlarged cysts. Therefore, RAS plays an important role in the pathogenesis of ADPKD. The polymorphisms of the RAS may be good candidates as disease-modifying genetic factors in ADPKD. In several cumulative renal survival analytical reports, it has been suggested that pa-

tients with ADPKD, homozygous for the D allele of the ACE gene, are at an increased risk of developing ESRD.[21-23] However, the effects of the polymorphisms of the AGT and ATR genes in ADPKD are uncertain.

We found no difference in the prevalence of hypertension or age at onset of ESRD in relation to the AGT M235T and ATR A1166C polymorphisms. This suggests that the AGT and ATR gene polymorphisms have no effect on the hypertension or ESRD in ADPKD. According to meta-analysis studies, the AGT M235T polymorphism has a weak association with essential hypertension in Caucasians, but has no association with cardiovascular and renal complications. From our data, the AGT M235T and ATR A1166C polymorphisms showed no association with hypertension or renal complications in ADPKD.

Our results are a little different from those of the meta-analysis. This can be explained as follows; 1) there may be some effects of racial genetic differences, as the distribution of the genotypes in Koreans are largely different from those in Caucasians. The frequencies of M235T and A1166C alleles were different in relation to ethnic group. The frequency of T235 allele was higher in Koreans ($\approx$0.8) than in Caucacians[12,24] ($\approx$0.4), but was similar with those in Chinese and Japanese.[25,26] Only a few studies are available concerning the ATR A1166C polymorphism. The frequency of the C1166 allele was lower in Koreans ($\approx$0.05) than in Caucacians[27] ($\approx$0.3); 2) the role of the RAS, or gene polymorphism, in hypertension may be different in essential hypertension and ADPKD. Hypertension is a heterogeneous and multifactorial disorder; 3) a crucial point in association studies is the sample size. The AGT M235T polymorphism, inform the meta-analysis study, has a weak association with hypertension. It may be possible that there is no association in small sample sizes. We analyzed 108 subjects with ADPKD and 105 normotensive controls. The frequency of the C1166 allele was too low ($\approx$0.05) to evaluate any association. Despite our small sample size, the results indicate that the AGT and ATR gene polymorphisms have no effect on the hypertension or ESRD in ADPKD. Even though the AGT M235T polymorphism has a weak association with hypertension, from the

meta-analytical data, degree of significance is likely to be very small.

In conclusion, there were no differences in the prevalence of hypertension and the age at onset of ESRD in relation to the AGT M235T and ATR A1166C polymorphisms. We suggest that the AGT and ATR gene polymorphisms would not have an effect on the hypertension and ESRD in ADPKD; further studies will be required in order to find the modifying gene in ADPKD.

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