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Research Article

Genetic variability in the renin-angiotensin system may modify renal responses to injury and disease progression. We examined whether the M235T polymorphism of the angiotensinogen (AGT) gene, the insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene, and the A1166--> C polymorphism of the angiotensin II type 1 receptor gene may be associated with disease progression in 168 Caucasian patients with IgA nephropathy. All patients had serial measurements of their creatinine clearance, proteinuria, and blood pressure (mean+/-SD) with a follow-up of 6.1+/-4.7 yr. The genotype frequencies for each gene were consistent with Hardy-Weinberg equilibrium, and were similar to those of 100 Caucasian control subjects. We examined two primary outcomes: (a) the rate of deterioration of Ccr, and (b) the maximal level of proteinuria. We found that patients with the AGT MT (n = 79) and TT (n = 29) genotypes had a faster rate of deterioration of Ccr than those with the MM (n = 60) genotype (i.e., median values, -6.6 and -6.2 vs. -3. 0 ml/min/yr, respectively; P = 0.01 by Kruskal-Wallis test). Similarly, patients with AGT MT and TT genotypes had higher maximal values of proteinuria than those with the MM genotype (i.e., median values, 2.5 and 3.5 vs. 2.0 g/d, respectively; P < 0.02 by Kruskal-Wallis test). Neither the ACE insertion/deletion nor angiotensin II type I A1166--> C gene […]

Association of Angiotensinogen Gene T235 Variant with Progression of Immunoglobin A Nephropathy in Caucasian Patients

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Abstract

Genetic variability in the renin–angiotensin system may modify renal responses to injury and disease progression. We examined whether the M235T polymorphism of the angiotensinogen (AGT) gene, the insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene, and the $A^{1166}\rightarrow C$ polymorphism of the angiotensin II type 1 receptor gene may be associated with disease progression in 168 Caucasian patients with IgA nephropathy. All patients had serial measurements of their creatinine clearance, proteinuria, and blood pressure (mean±SD) with a follow-up of 6.1±4.7 yr. The genotype frequencies for each gene were consistent with Hardy-Weinberg equilibrium, and were similar to those of 100 Caucasian control subjects. We examined two primary outcomes: (*a*) the rate of deterioration of Ccr, and (*b*) the maximal level of proteinuria. We found that patients with the AGT MT ($n = 79$) and TT ($n = 29$) genotypes had a faster rate of deterioration of Ccr than those with the MM ($n = 60$) genotype (i.e., median values, −6.6 and −6.2 vs. −3.0 ml/min/yr, respectively; $P = 0.01$ by Kruskal-Wallis test). Similarly, patients with AGT MT and TT genotypes had higher maximal values of proteinuria than those with the MM genotype (i.e., median values, 2.5 and 3.5 vs. 2.0 g/d, respectively; $P < 0.02$ by Kruskal-Wallis test). Neither the ACE insertion/deletion nor angiotensin II type I $A^{1166}\rightarrow C$ gene polymorphism was associated with disease progression or proteinuria in univariate analysis. Multivariant analysis, however, detected an interaction between the AGT and ACE gene polymorphisms with the presence of ACE/DD polymorphism adversely affecting disease progression only in patients with the AGT/MM genotype ($P = 0.008$). Neither of these gene polymorphisms was associated with systemic hypertension. Our results suggest that polymorphisms at the AGT and ACE gene loci are important markers for predicting progression to chronic renal failure in Caucasian patients with IgA nephropathy. (*J. Clin. Invest.* 1997. 100:814–820.) Key words: renin–angiotensin system • genetic association • IgA nephropathy

Introduction

IgA nephropathy, the most common glomerulonephritis worldwide, is an important cause of end-stage renal disease (1). Although the factors responsible for progressive renal failure in this disease have not been fully elucidated, systemic hypertension and increased urinary protein excretion are clinical predictors of poor outcome (1, 2). Recent studies on IgA nephropathy have demonstrated intrarenal angiotensin II hyperreactivity in patients with progressive disease (3), while treatment with angiotensin-converting enzyme (ACE)[1] inhibitors has been shown to improve glomerular permselectivity (4) and to delay progression to chronic renal failure (5, 6). Taken together, these studies suggest that systemic and/or intrarenal activation of the renin–angiotensin system may be an important mechanism for promoting progressive renal failure in this disease.

Polymorphisms of the genes encoding the components of the renin–angiotensin system have been identified recently, and their relationship to clinical disease has been examined (7–24). For example, the M235T polymorphism of the angiotensinogen gene (7–10) and the $A^{1166}\rightarrow C$ polymorphism of the angiotensin II type 1 (ATR_1) receptor gene (11) have been found to be associated with essential hypertension, while the insertion/deletion (I/D) polymorphism of the ACE gene has been found to be associated with cardiovascular diseases (12–14) and renal disease progression (15–20). Not all of these associations, however, could be replicated consistently (21–24). In this study, we tested the hypothesis that genetic variability in the renin–angiotensin system may modify renal disease progression in IgA nephropathy. Specifically, we tested whether the M235T polymorphism of the angiotensinogen gene, the I/D polymorphism of the ACE gene, and the $A^{1166}\rightarrow C$ polymorphism of the ATR_1 gene may be associated with disease progression in Caucasian patients with IgA nephropathy.

Methods

Study patients and control subjects. The study patients were recruited through the Toronto Glomerulonephritis Registry, a regional research network that follows all cases of biopsy-proven glomerulonephritis from 32 academic and 18 community nephrologists, covering a population of 4.5 million (25). Between March of 1994 and April of 1996, 220 Caucasian patients with primary IgA nephropathy had been followed actively by the Toronto Glomerulonephritis Registry since their renal biopsy. 168 patients who had a minimal follow-up of 2 yr, and who gave informed consent, were recruited into this study. All patients had serial measurements of their urinary creatinine clearance (Ccr), proteinuria, and blood pressure. Serum and urine creatinine concentrations were determined by the Autoanalyzer method (Technicon Inc., Chaucy, NY), and urinary protein concentration was

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1. *Abbreviations used in this paper:* ACE, angiotensin-converting enzyme; ATR_1, angiotensin II type 1, Ccr, creatinine clearance; I/D, insertion/deletion; s-Cr, serum creatinine.

determined by the Folin-Lowry method. Urinary Ccr was calculated using simultaneous measurements of serum and 24-h urinary creatinine, and standardized to a body surface area of 1.73 m^2 (26). To provide the local genotype frequencies for the candidate genes being examined, 100 healthy Caucasian subjects with no history of renal disease or hypertension were also recruited. The research protocol used for this study was approved by the University of Toronto Human Subjects Review Committee.

Outcome definitions. To increase the sensitivity of detecting an association, three patient subgroups were segregated according to their rate of deterioration of Ccr over time: S, slow progressors whose rate of deterioration of Ccr was less than or equal to 3 ml/min/yr; I, intermediate progressors whose rate of deterioration of Ccr was greater than 3 but less than or equal to 6 ml/min/yr; and F, fast progressors whose rate of deterioration of Ccr was greater than 6 ml/min/yr. Similarly, the maximal level of proteinuria that persisted for at least 6 mo during the observed course was used to segregate the study patients into three groups: M, mild proteinuria with maximal proteinuria less than or equal to 1.5 g/d; Mod, moderate proteinuria with maximal proteinuria greater than 1.5 but less than or equal to 3 g/d; and S, severe proteinuria with maximal proteinuria greater than 3 g/d.

Extraction of genomic DNA and genotype determinations. Genomic DNA was extracted from peripheral blood lymphocytes by the salting-out method (27). The Met^{235}→Thr polymorphism in exon 2 of the angiotensinogen gene was determined according to the method of Russ et al. (28). PCR amplification yielded a 165-bp product, which was digested overnight with AspI. After restriction digest, the PCR fragment containing the Met variant remained the same size, while the fragment containing the Thr variant was cut into 141- and 24-bp fragments. When the digested products were resolved by electrophoresis on a 2.5% agarose gel containing ethidium bromide, the Met and Thr variants could be scored accordingly. The I/D polymorphism in intron 16 of the ACE gene was determined according to the method of Rigat et al. (29), except that 5% (vol:vol) DMSO was added to the PCR reaction to minimize mistyping of the ID genotype as DD (30). PCR amplification yielded a fragment with (I allele) or without the insertion allele (D allele) of ~ 490 and 190 bp, respectively. All samples that were typed as DD were retyped with the insertion allele–specific primers, using the method of Lindpaintner et al. (21). With these primers, PCR amplification yielded a 335-bp product when there was mistyping, and no product when the DD typing was correct. All PCR products were resolved by electrophoresis on a 1% agarose gel. The A^{1166}→C polymorphism in the 3′ untranslated region of the ATR_1 gene was determined using the method of Doria et al. (31). PCR amplification yielded a 546-bp product which was digested with DdeI overnight. The size of the 1166A allele remained unchanged after restriction digest, while the fragment containing the 1166C allele was cut into 435- and 111-bp fragments. After resolving on a 2% agarose gel by electrophoresis, these alleles were scored accordingly. Positive controls consisting of DNA samples from subjects of known genotypes and a negative control of complete PCR reaction mix without DNA were included in each PCR reaction.

Statistical analyses. All data are presented as mean±SD or percent. Student's *t* test or ANOVA was used for analysis of continuous variables, and χ^2 test was used for categoric variables. For data that were not normally distributed, the Kruskal-Wallis test was used. Linear regression analysis was performed to generate the rate of deterioration of Ccr over time with an average of 10 data points used for each linear regression. The mean correlation coefficients (r) of the rate of deterioration of Ccr over time for the fast, intermediate, and slow progressors were 0.89, 0.84, and 0.51, respectively. For each candidate gene marker, allele frequencies were calculated from the genotype frequencies in the patients and control subjects. The expected number of each genotype was obtained by multiplying the Hardy-Weinberg frequencies by the sample size. Deviation from Hardy-Weinberg equilibrium was assessed by goodness-of-fit between the observed and expected numbers using χ^2 test with 1 degree of freedom (df). Differences in the genotype distributions between patients with different rates of deterioration of Ccr over time or proteinuria were tested by χ^2 test with 2 df. Odds ratios and 95% confidence interval were used for estimating the risk of association between a primary outcome category and a specific genotype. To further delineate the relationships among the above gene polymorphisms, hypertension, and the rate of loss of renal function, multiple logistic regression analysis was performed (32). The categoric variable of fast, intermediate, and slow progressors was used as a response variable, and age (at the last follow-up), sex, serum creatinine at presentation, mean arterial blood pressure at presentation and at last follow-up, and the three candidate gene polymorphisms (AGT MM vs MT/TT; ACE DD vs. ID/II; and ATR_1 CC vs. AC/AA) were tested as independent variables. In addition, two-way interactions of these latter variables were also tested. All statistical analyses were performed using statistical packages (Instat v.2; GraphPAD Software for Science, San Diego, CA; or SAS (SAS Institute Inc., Cary, NC).

Table I. Clinical Characteristics of Patients with Different Rates of Loss of Renal Function

	S	I	F	*P* value
	n = 52	*n = 44*	*n = 72*	
Current age (yr)	51±14	53±13	46±13	= 0.015
Sex (male:female)	2.7:1.0	2.4:1.0	1.8:1.0	= 0.52
Follow-up time (yr)	8.6±5.2	6.0±4.9	4.1±3.2	< 0.0001
s-Cr at presentation (mg/dl)	1.4±0.42	2.3±1.7	2.3±1.8	< 0.002
s-Cr at last follow-up (mg/dl)	1.6±0.68	7.3±4.5	8.8±4.2	< 0.0001
Slope of Cr clearance over time (ml/min/yr)	−0.60±4.6	−3.9±0.90	−12.6±8.4	< 0.001
Maximum proteinuria* (g/d)	2.0±1.8	3.1±2.2	4.3±3.1	< 0.0001
MABP‡ at presentation (mmHg)	100±20	100±20	110±20	< 0.007
MABP at last follow-up (mmHg)	100±10	110±20	110±20	< 0.004
Antihypertensive Rx§	56%	68%	82%	< 0.002
With ACE inhibitor	40%	38%	43%	= 0.80
With multiple antihypertensive drugs	20%	28%	34%	= 0.52

S, slow progressors; rate of deterioration of Ccr ≤ 3 ml/min/yr. I, intermediate progressors; 3 ml/min/yr < rate of deterioration of Ccr ≤ 6 ml/min/yr. F, fast progressors; rate of deterioration of Ccr > 6 ml/min/yr. *Maximal level of proteinuria that persisted for at least 6 mos; ‡mean arterial blood pressure; §anytime during the patient course.

Results

Patient characteristics. 168 patients were studied. Their current age was 47±14 yr, and their mean follow-up time was 6.1±4.7 yr. 50% of the patients had s-Cr concentrations > 5 mg/dl at their last follow-up, and 70% of the patients required treatment with one or more antihypertensive medications during their course. Table I shows the clinical characteristics of the three patient subgroups segregated by their rate of loss of renal function. Not surprisingly, the fast progressors were younger and had the highest s-Cr, proteinuria, and mean arterial blood pressure at presentation and last follow-up. In contrast, the slow progressors had the lowest serum creatinine (s-Cr), proteinuria, and mean arterial blood pressure. Such a classification allowed separation of the study patients into three groups with graded disease severity.

Genotype frequencies. We found that the distribution of genotypes for each candidate gene was similar between the study patients and control subjects (Table II). Furthermore, the expected frequencies of the angiotensinogen genotypes (MM:MT:TT = 59:81:28), the ACE genotypes (DD:ID:II = 55:82:31), and ATR_1 genotypes (AA:AC:CC = 84:70:14), under the assumption of Hardy-Weinberg equilibrium, did not differ from the observed frequencies in our patients.

Genotype associations with clinical outcomes. We examined if the distributions of the genotypes in each candidate gene differed between three patient subgroups segregated on the basis of their rates of loss of renal function. We found an excess of angiotensinogen MT and TT genotypes in the moderate and fast progressor groups (Fig. 1 *A*). Moreover, these associations best fit a dominant genetic model in which both 235T homozygotes and heterozygotes conferred the same risk for moderate to severe deterioration in renal function (Fig. 2 *A*). Specifically, the odds ratio of the MT and TT genotypes (as compared to the MM genotype) was 1.7 (95% confidence interval: 0.76–3.8) in the moderate vs. slow progressor group, and 3.8 (95% confidence interval: 1.7–8.2) in the fast vs. slow progressor group. In absolute terms, patients with MT (n = 79) and TT (n = 29) genotypes had more rapid loss of renal function than those with the MM (n = 60) genotype (i.e., medians: −6.6 and −6.2 vs. −3.0 ml/min/yr, respectively; P = 0.01 by Kruskal-Wallis test).

Similarly, we observed an excess of angiotensinogen MT and TT genotypes in patients with moderate and severe proteinuria (Fig. 1 *B*), and this association also fit best under a dominant genetic model (Fig. 2 *B*). The odds ratio for the MT/TT genotypes was 1.3 (95% confidence interval: 0.59–2.9) in patients with moderate vs. mild proteinuria and 3.0 (95% confidence interval: 1.3–6.7) in patients with severe vs. mild proteinuria. Patients with MT and TT genotypes had higher values of their maximal proteinuria than those with the MM genotype (i.e., medians: 2.5 and 3.5 vs. 2.0, respectively; $P <$ 0.02 by Kruskal-Wallis test).

In univariate analysis, neither the I/D polymorphism of the ACE gene nor the $A^{1166}\rightarrow C$ polymorphism of the ATR_1 gene

Table II. Genotype Frequencies in Caucasian IgA Patients and Controls

	Patients	Controls
	n = 168	*n = 100*
AGT genotypes		
MM	60 (36%)	34 (34%)
MT	79 (47%)	50 (50%)
TT	29 (17%)	16 (16%)
	χ^2 (2 df) = 0.23; P = 0.89	
ACE genotypes		
DD	55 (33%)	30 (30%)
ID	81 (48%)	49 (49%)
II	32 (19%)	21 (21%)
	χ^2 (2 df) = 0.28; P = 0.87	
ATR_1 genotypes		
AA	82 (49%)	56 (56%)
AC	73 (43%)	37 (37%)
CC	13 (8%)	7 (7%)
	χ^2 (2 df) = 1.3; P = 0.52	

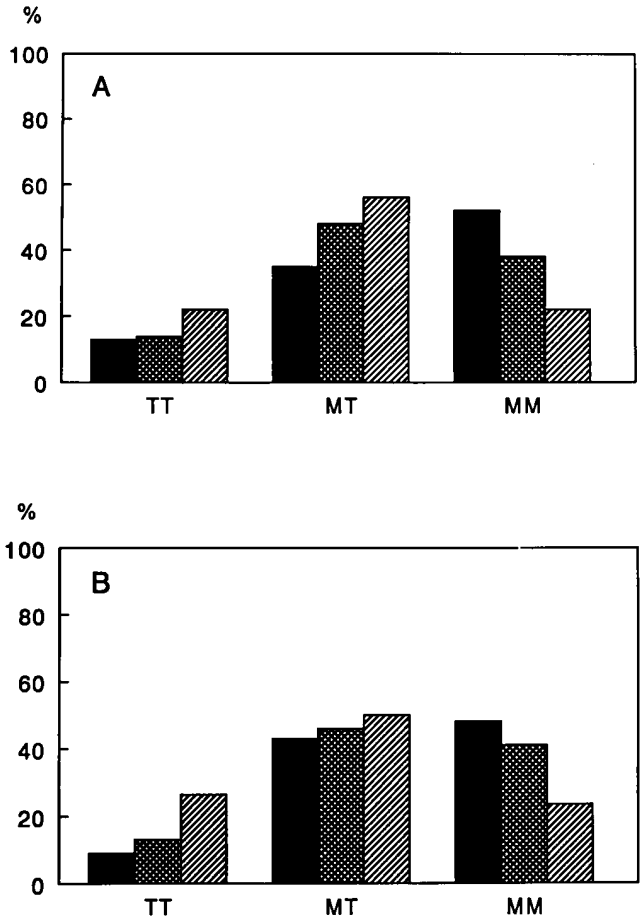

Figure 1. (*A*) AGT genotype frequencies in slow, moderate, and fast progressors. The distributions of the TT, MT, and MM genotypes were significantly different between these three patient subgroups segregated by disease progression rates (χ^2 [4df] = 12.2; P = 0.016). *Black bars*, slow (n = 52); *dotted bars*, moderate (n = 44); *striped bars*, fast (n = 72). (*B*) AGT genotype frequencies in patients with mild, moderate, and severe proteinuria. The distributions of the TT, MT, and MM genotypes were significantly different between these three patient subgroups segregated by the severity of proteinuria (χ^2 [4df] = 11.1; P = 0.025). *Black bars*, mild (n = 44); *dotted bars*, moderate (n = 56); *striped bars*, severe (n = 68).

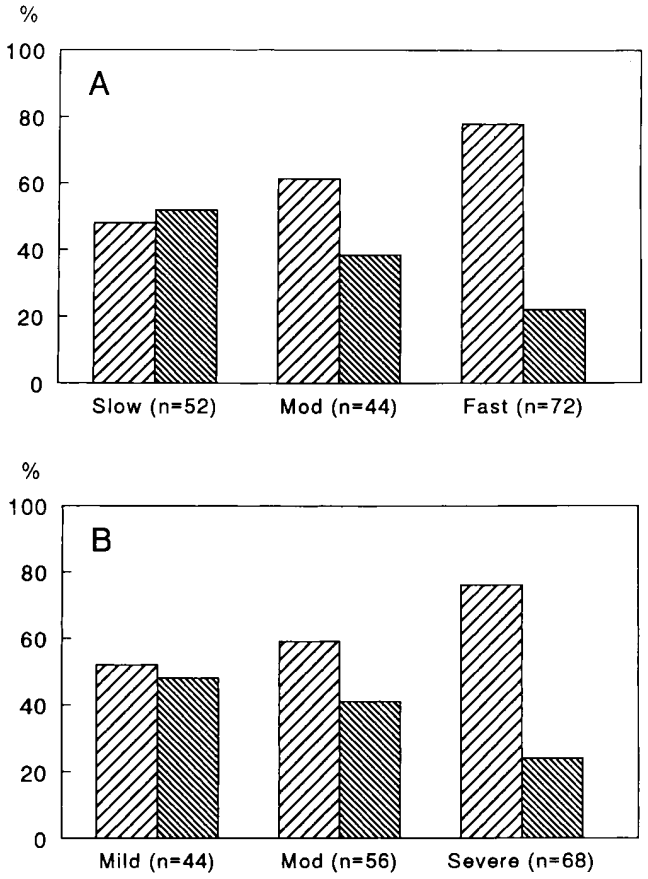

Figure 2. (*A*) AGT genotype frequencies in slow, moderate (*Mod*), and fast progressors under a dominant genetic model. The MT/TT genotypes were significantly increased in patients in the moderate and fast progression groups (χ^2 for trend [1df] = 11.8; $P = 0.0006$). (*B*) AGT genotype frequencies in patients with mild, moderate (*Mod*), and severe proteinuria under a dominant genetic model. The MT/TT genotypes were significantly increased in patients with moderate and severe proteinuria (χ^2 for trend [1df] = 11.8; $P < 0.007$). *Wide stripes*, MT/TT; *narrow stripes*, MM.

Table III. Primary Outcomes of Patients by ACE and ATR_1 Genotypes

	ΔCcr over time	Maximal proteinuria
	ml/min/yr	*gld*
ACE genotypes		
DD ($n = 55$)	−6.6±7.8	3.4±2.7
ID ($n = 81$)	−6.0±8.4	3.0±2.8
II ($n = 32$)	−6.6±7.2	3.6±2.5
	(F = 0.12, $P = 0.89$)	(F = 0.69, $P = 0.5$)
ATR_1 genotypes		
AA ($n = 82$)	−4.7 (−1.7, −8.9)*	3.3±2.6
AC ($n = 73$)	−4.0 (−2.4, −7.6)	3.1±2.2
CC ($n = 13$)	−4.2 (−3.0, −7.2)	3.8±2.3
	(KW = 0.28, $P = 0.87$)‡	(F = 0.49, $P = 0.61$)

*Data expressed as medians and interquartile range. ‡Kruskal-Wallis test; all other comparisons were tested by ANOVA.

Discussion

In this study, we tested the hypothesis that genetic variability of the renin–angiotensin system may modify the progression rate to chronic renal failure in IgA nephropathy. We postulated that the progression of this disease is a complex trait, influenced by multiple genetic and environmental factors (33). We found that our patients with one or both copies of the angiotensinogen T235 alleles had a faster rate of decline in their renal function than patients homozygous for the M235 allele. Previous studies have shown that plasma angiotensinogen concentrations correlate with blood pressure, and are higher in hypertensive patients as compared to normotensive subjects (7). Several family-based studies of hypertensive patients have detected linkage of the angiotensinogen locus to essential hypertension and preeclampsia (7–10). In the larger study (7), two coding sequence variants, M235T and T174M, have been

was associated with disease progression or proteinuria in our patients. Their rate of loss of renal function and maximal proteinuria segregated by the ACE and ATR_1 genotypes are shown in Table III.

Multivariant analysis of risk factors for renal functional deterioration. To further delineate the relationship between the AGT MT/TT polymorphisms and the effects of blood pressure on the rate of loss of renal function, multiple logistic regression was performed. Patient age (odds ratio = 1.04 for each 1 yr age difference between patients; $P = 0.0005$), serum Cr at presentation (odds ratio = 1.46 for each 1 mg/dl difference between patients; $P = 0.005$) and the AGT MT/TT vs. MM genotype (odds ratio = 2.86; $P < 0.001$) were identified as independent variables associated with progressive loss of renal function. Mean arterial blood at presentation or at last follow-up did not predict renal function deterioration. Of interest, an interaction was detected between the ACE and AGT gene polymorphisms. The ACE/DD polymorphism adversely affected disease progression only in patients with the AGT/MM genotype ($P = 0.008$) (Fig. 3).

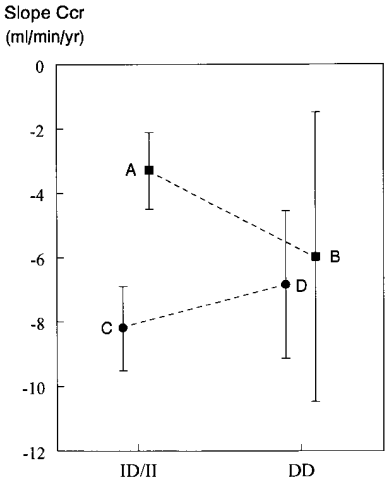

Figure 3. Interaction between the AGT and the ACE gene polymorphisms on the rates of loss of renal function. The rates of loss of renal function (mean±95% confidence interval) in the study patients are segregated into four genotype combinations: (*A*) AGT MM and ACE ID/II ($n = 44$); (*B*) AGT MM and ACE DD ($n = 16$); (*C*) AGT MT/TT and ACE ID/II ($n = 69$); and (*D*) AGT MT/TT and ACE DD ($n = 39$). The presence of an interaction is indicated by the deviation of line AB from being paralleled to line CD ($P = 0.008$). The ACE/DD polymorphism appeared to modulate adversely the loss of renal function only in patients with the AGT/MM genotype.

shown to be associated with hypertension. Only the 235T variant, however, is associated with increased plasma angiotensinogen concentrations (7). Since plasma angiotensinogen concentrations are close to the K_m of renin, a rise or fall in angiotensinogen can lead to a parallel change in the formation of angiotensin II, promoting the development of hypertension (7, 34). Indeed, recent findings in a transgenic mouse model of hypertension, in which variation in blood pressure correlates with the number of angiotensinogen gene copies (i.e., from 1–4) and plasma angiotensinogen concentrations, support the above hypothesis (35). Thus, the angiotensinogen T235 variant might be a risk factor for progressive IgA nephropathy through its association with hypertension. To further delineate the relationship between the angiotensinogen T235 variant and systemic blood pressure, and also their effects on the rates of loss of renal function, we examined these variables in a multivariant analysis. While the angiotensinogen 235T allele remained an independent risk factor predicting progressive loss of renal function, we were unable to detect any association between the angiotensinogen 235T variant and systemic blood pressure. Thus, our findings were similar to the results of a recent study in which the angiotensinogen T235 variant was found to be associated with diabetic nephropathy, but not hypertension (36).

The mechanism(s) responsible for the effect(s) of the angiotensinogen T235 variant on progression of IgA nephropathy remain speculative at this time. One limitation in the current study is that a large proportion of our patients were treated with antihypertensive medications, and pretreatment blood pressure measurements were not available in many of them. As shown in Table I, 56–82% of our patients in the three patient group received antihypertensive treatment. In addition, 40% of our patients were treated with an ACE inhibitor, and one-third were treated with multiple antihypertensive drugs. Thus, antihypertensive treatment might have limited our ability to detect an association between the angiotensinogen T235 variant and hypertension, and it remains possible that the influence of this gene variant may be related to an underlying effect on blood pressure. On the other hand, at least two other mechanisms may potentially explain the above associations. The angiotensinogen T235 variant (as compared to the homozygous M235 variant) might be associated with an increase in intrarenal angiotensinogen production. Since all the components of the renin–angiotensin system are present in the kidney (37), an increase in intrarenal angiotensin II production could contribute to or amplify renal injury (38, 39), an effect that might not be reflected in the systemic circulation. In this context, de novo angiotensinogen expression has recently been shown to occur in the endothelial and mesangial cells of the rat remnant glomerulus, especially during the phase of evolving sclerosis (40). If this response were modified by the angiotensinogen M235T polymorphism in humans, then it may provide a mechanistic explanation for the association of the T235 variant with proteinuria and progressive renal failure, independent of systemic hypertension. Finally, it is possible that the T235 variant may merely be a marker in linkage disequilibrium with a close by common gene variant, and that such a putative gene variant might influence renal disease progression but not blood pressure.

Previous studies have documented that the D/D polymorphism of the ACE gene is associated with increased plasma ACE concentrations (12), cardiac diseases such as myocardial infarction and left ventricular hypertrophy (12, 14), and progression of diabetic nephropathy (15, 16). These associations, however, have not been consistently replicated (21, 22, 24). In this study, we did not detect any association between the ACE D/D polymorphism and renal functional deterioration in univariate analysis. An interaction was detected, however, in multivariant analysis, suggesting that the ACE D/D polymorphism may adversely modify the rates of loss of renal function only in patients with the angiotensinogen MM genotype. Including the present study, six studies have now examined the association of the ACE D/D polymorphism with progression of IgA nephropathy (17–20, 23). As shown in Table IV, four studies have detected an association, while two have not. We believe that the discrepancies in the above studies and between our univariate and multivariate analyses may be in part related to sample size. This problem is best exemplified by recent studies on the genetic susceptibility of diseases such as schizophrenia, diabetes, and multiple sclerosis, where replication of results by different groups has been inconsistent (41, 42). While spurious associations from comparing multiple markers in the whole genomic scanning may contribute to this problem (41), Bell and Lathrop have recently emphasized that the failure to detect a true susceptibility locus by different studies can occur as a result of inadequate power (42). For example, assuming that four loci, each with moderate effect, contribute to a disease susceptibility, 86 affected sib-pairs are required to detect one susceptibility locus. To replicate the same result in a second study, however, would require twice the sample size, and to detect all four loci in a single study would require three times the sample size of the first study (42). For a multifactorial disease with several major susceptibility loci, the expected sample size required to replicate linkage/association to a specific locus in a second study will be very much larger than that of the first study, where any of the loci are available for detection. Thus, none of the six studies on IgA nephropathy likely has sufficient power to detect multiple genetic markers associated with disease progression.

An important issue arising from this study is whether the clinical course of patients with the above adverse genotypes can be modified by treatment with an ACE inhibitor as com-

Table IV. Summary of Recent Association Studies on ACE D/D Polymorphisms and Progression of IgA Nephropathy

n	Race	ACE(D/D)	D/D:I/D:I/I	HWE*	Reference
48	Japanese	Positive	17:27:56	$\chi^2 = 6.3$ $P < 0.02$	20
53	Japanese	Positive	30:32:38	$\chi^2 = 6.7$ $P < 0.01$	19
122	Caucasian	Negative	39:40:21	$\chi^2 = 6.8$ $P < 0.01$	23
64	Caucasian	Positive	14:59:27	$\chi^2 = 2.7$ $P \sim 0.10$	18
100	Caucasian	Positive	40:41:19	$\chi^2 = 2.7$ $P > 0.2$	17
168	Caucasian	Negative	33:48:19	$\chi^2 = 0.02$ $P > 0.9$	Current study

*Hardy-Weinberg equilibrium (HWE) in the total patient population ($P < 0.05$ indicates that the study patient population is not in HWE).

pared to other antihypertensive medications. Because of the limitation of the current study design, however, which is retrospective and observational, we were unable to address this question. Many of our patients were treated with antihypertensive medications at different time points, with different doses, and for varying durations. Further, a number of them were treated with multiple antihypertensive medications. Finally, our study patients were managed by a number of different nephrologists without a standardized protocol so that there were no uniform blood pressure treatment target goals. Thus, we were unable to compare specific antihypertensive drug treatments.

In conclusion, our study suggests that the T235 variant of the angiotensinogen gene is a marker associated with progressive loss of renal function in Caucasian patients with IgA nephropathy. Future studies with larger patient sample size will be needed to confirm both the angiotensinogen 235T and ACE D/D associations. Moreover, controlled clinical trials will be needed to test whether the clinical course of patients with these genetic risk markers can be modified by pharmacologic interventions (e.g., treatment with an ACE inhibitor or angiotensin II receptor antagonist).

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