Association of Angiotensin II Type 2 Receptor Gene A1818T Polymorphism with Progression of Immunoglobulin A Nephropathy in Korean Patients

We determined the relationship between the progression of immunoglobulin A nephropathy (IgAN) and the A1818T polymorphism in intron 2 of Angiotensin II type 2 receptor (AT2R) gene, which might play protective roles in the pathogenesis of IgAN. Patients with biopsy-proven IgAN were recruited from the registry of the Progressive REnal disease and Medical Informatics and gEnomics Research (PREMIER) which was sponsored by the Korean Society of Nephrology. A1818T polymorphism of AT2R gene was analyzed with PCR-RFLP method and the association with the progression of IgAN, which was defined as over 50% increase in baseline serum creatinine level, was analyzed with survival analysis. Among the 480 patients followed for more than 10 months, the group without T allele had significantly higher rates of progression of IgAN than the group with T allele (11.4% vs. 3.9%, p=0.024), although there were no significant differences in the baseline variables such as initial serum creatinine level, the degree of proteinuria, and blood pressure. In the Cox's proportional hazard model, the hazard ratio of disease progression in the patients with T allele was 0.221 (95% confidence interval for Exp(B): 0.052-0.940, p=0.041) compared to that of without T allele. In conclusion, A1818T polymorphism of AT2R gene was associated with the progression of IgAN.

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INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is the most common type of primary glomerulonephritis in the world among patients undergoing renal biopsy. It is known to be an important cause of end stage renal disease. The actuarial renal survival at 10 yr in adults with IgAN is about 80-85% according to several epidemiologic studies of Asian countries (1-3). Although several clinical factors such as severe proteinuria, arterial hypertension, elevated serum creatinine level (4-6), and the severity of glomerular sclerosis and interstitial fibrosis on the renal pathology (7) have been thought to be related to the progression of IgAN, it is not clear why some of patients develop progressive disease, while the others do not. It has been suggested that genetic factors may contribute not only to the susceptibility to IgAN but also to its progression. There have been so many genetic association studies on the candidate genes of IgAN progression, of which the most widely studied have been polymorphisms of genes involved in the renin-angiotensin system (RAS), including

insertion/deletion (I/D) polymorphisms of angiotensin converting enzyme (ACE) gene (10), and angiotensinogen gene polymorphisms (11). However, results of these studies were inconsistent.

Several experimental studies have suggested that as an another important part of the RAS, counter-regulatory to the vasoconstrictor action of the angiotensin II via AT1R, angiotensin II type 2 receptor (AT2R) might have a protective role in the ischemic kidney injury (12) and that tubular expression of AT2R and imbalance to AT1R could be related to the pathogenesis of IgAN (13). Additionally, according to recent association study in type 1 diabetic patients, among the polymorphisms of X-chromosomal AT2R gene, A1818T polymorphism was reported to be related to the kidney function (14). Therefore, we hypothesize that A1818T polymorphism of AT2R gene may be involved in the progression of IgAN.

MATERIALS AND METHODS

Study subjects

This study was approved by the Institutional Review Board in Seoul National University Hospital and other participated hospitals before gathering the data. Informed consents were obtained from the patients. The subjects were enrolled in the Progressive REnal disease and Medical Informatics and gEnomics Research (PREMIER) which was sponsored by the Korean Society of Nephrology since August 2003. In PREMIER study, 34 hospitals and clinics in Korea participated. We analyzed the clinical data of 4,406 patients aged 15 yr or more with glomerulonephritis diagnosed by renal biopsy from May 1982 to December 2006. From this registry, 480 patients with primary IgAN, who were over 15 yr old, had a minimum follow up period of 10 months were enrolled in this study. Diagnosis of IgAN was based on established pathologic criteria, including mesangial expansion and the diagnostic presence of IgA as the sole, predominant, or co-dominant immunoglobulin. Patients with evidence of systemic diseases, such as diabetes, chronic liver disease, or systemic lupus erythematosus, were excluded. One hundred healthy, randomly selected, age-matched, normotensive subjects without any evidence of renal disease were also recruited from the Health Promotion Centre of Seoul National University Hospital as control.

Clinical data

The participated researchers had selected candidate patients, and one qualified nurse visited every participated institution to collect the clinical data at the time of renal biopsy and during follow-up visits. We gathered the data of age, gender, body mass index (BMI), blood pressure, serum protein level, serum albumin level, serum cholesterol level, serum uric acid level, serum creatinine level, the degree of proteinuria by spot urine protein creatinine ratio (suPCR) at the time of renal biopsy and medication of RAS inhibitors or steroid during follow-up period. The hypertension was defined as systolic blood pressure (SBP) 140 mmHg or higher, diastolic blood pressure (DBP) 90 mmHg or higher, or taking antihypertensive medication before renal biopsy and the severe proteinuria were defined as more than 3 of suPCR, respectively. The estimated glomerular filtration rate (eGFR) was calculated by the modified MDRD equation (15).

Definition of disease progression

The primary end point was an increase of serum creatinine level by more than 50% of baseline value or initiation of renal replacement therapy for the end stage renal disease. We defined the progressive disease as the group of patients of IgAN reached to the primary end point and stable disease for the others. The follow-up duration was the duration of time between renal biopsy and the primary end point or the last follow-up date.

Extraction of genomic DNA and genotype determination

Genomic DNA was isolated from peripheral blood using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, U.S.A.), according to the manufacturer's instructions. Genotyping for the A1818T polymorphism of AT2R gene was done using methods previously described (16). Briefly for the region spanning the polymorphism, the minisequencing primers were 5′-CTGTATTTTGCAAAACTCCT-3′ and 5′-TTATGTTAATTTGTTAGGTC-3′, respectively. The resulting amplicon was digested using Sau961 restriction enzyme (Promega), which recognizes the 1818T allele. The restriction products were then visualized on 2% agarose gels containing ethidium bromide. Confirmation of genotypes was done by direct DNA sequencing using the ABI Prism Big Dye Terminator kit (Applied Biosystems, Foster City, CA, U.S.A.) of 10% of the study population samples. In all cases, the genotype determined by PCR restriction fragment length polymorphism assay was identical to that determined by DNA sequencing.

Statistical analysis

All data were analyzed using SPSS 12.0 KO for Windows. Mantel-Haenszel chi-square test was used to detect the differences in the genotype distributions of the patients and controls. The Continuous variables were tested by Student's t-test or Mann-Whitney tests and the discrete variables by chi-square test. To test the significance of various prognostic factors related to the disease progression, survival analysis using the log rank test and the Cox's proportional hazards

models were carried out. With the Kaplan-Meier analysis, we expressed survivorship curve about the difference between patients with T allele and those without T allele. And then we analyzed the multivariate analysis with Cox's model, adjusted for age, sex, serum creatinine level, the degree of proteinuria (as a continuous variables, and a discrete variable suPCR <3 or $\geq$3), the presence of hypertension, and decreased renal function at the time of diagnosis.

RESULTS

Genetic distribution in control group and IgAN patient group

We investigated A1818T polymorphism in intron 2 of the AT2R gene located on X-chromosome. Table 1 shows the genotype distribution in control and patients groups. The genotype distribution was not different between control and patient groups (χ^2=5.111, p=0.078). This finding suggested that A1818T polymorphism of AT2R gene was not associated with susceptibility of IgAN. The genotype distribution in female control group and in female patient

group did not deviate from Hardy-Weinberg equilibrium (p>0.05).

Baseline characteristics of patients with IgAN

Among 480 patients with biopsy-proven IgAN, progressive disease was observed in 47 patients in 9.8% of the population after a follow-up of 30.33±3.34 months (mean ± standard deviation). There was a higher proportion of male patients in patients with progressive disease than in those with stable disease. The well-known indicators of poor renal function, such as lower eGFR and higher serum creatinine level, higher blood pressure, more severe proteinuria were observed in progressive disease group, compared to stable disease group (Table 2). The proportion of patients taking inhibitors of renin-angiotensin systems and that of patients with a history of steroid therapy were not different between 2 groups.

Comparison between patients with T allele and those without T allele

Males were less common in patients group with T allele than in patients group without T allele (38.8% vs. 57.8% respectively, p=0.001) (Table 3). Patients with the T allele were significantly younger at the time of kidney biopsy than those without it (33.36±12.68 vs. 36.61±13.92 respectively, p=0.025) (Table 3). Patients with T allele showed lower serum uric acid than those without it (5.65±2.01 vs. 6.13±1.75 respectively, p=0.033) (Table 3). The baseline renal function, blood pressure, and the proportion of patients taking inhibitors of RAS or steroid were not different between patients with the T allele and those without it. The progression of renal disease was observed in 11.4% (43/334) of pati-

Table 1. Genotyping of controls and patients with immunoglobulin A nephropathy (IgAN)

Genotype	Control* total	IgAN*		
		Total	Male	Female
AA	87 (87)	377 (78.5)	218 (84.5)	159 (71.6)
AT	10 (10)	58 (12.1)		58 (26.1)
TT	3 (3)	45 (9.4)	40 (15.5)	5 (2.3)
Total	100 (100)	480 (100)	258 (100)	222 (100)

*χ^2=5.111, p value=0.078, Data are N (%).

Table 2. Baseline characteristics of total patients with immunoglobulin A nephropathy

Variables	Total patients (N=480)	Stable disease (N=433)	Progressive disease (N=47)	p value
Follow-up duration (month)	30.33±3.34	28.84±18.68	44.74±28.05	<0.001
Age (yr)	35.91±13.72	35.74±13.89	37.58±11.95	0.227
Male (%)	53.8	51.7	73.3	0.007
BMI[†] (kg/m²)	23.43±3.34	23.44±3.36	23.27±3.14	0.94
SBP[‡] (mmHg)	126.14±19.33	125.30±18.40	135.37±26.14	0.041
DBP[§] (mmHg)	79.71±12.88	79.01±12.23	87.46±16.99	0.002
Hypertension (%)	42.5	40.9	64.3	0.018
eGFR* (mL/min/1.73 m²)	77.47±32.87	80.15±31.60	47.94±32.52	<0.001
eGFR <60 mL/min/1.73 m² (%)	29.3	25.7	77.4	<0.001
Serum creatinine (mg/dL)	1.30±1.25	1.19±0.95	2.40±2.59	<0.001
Spot urine PCR[‖]	1.97±3.03	1.90±3.01	2.86±3.20	0.001
Spor urine PCR[‖] >3 (%)	16.3	14.7	33.3	0.009
Serum_albumin (mg/dL)	3.82±0.65	3.85±0.65	3.50±0.56	0.002
Serum total cholesterol (mg/dL)	195.28±72.39	195.99±74.39	186.10±37.59	0.722
Serum uric acid (mg/dL)	6.04±1.81	5.92±1.74	7.83±1.98	<0.001
ACEI/ARB use (%)	65.3	66.2	55.3	0.213
Steroid use (%)	12.2	12.1	13.2	0.797

*estimated Glomerular Filtration Rate; [†]Body Mass Index; [‡]Systolic Blood Pressure; [§]Diastolic Blood Pressure; [‖]Protein Creatinine Ratio.

ents without the T allele and in 3.9% (4/103) of those with it (*p*=0.024) (Table 4). According to sex, there was no significant difference in the incidence of progressive renal disease between patients with the T allele and those without it. However, it tended to increase in the patients without the T allele (Table 4).

Table 3. Comparison between without T allele and with T allele

Variables	Without T allele* (N=377)	With T allele* (N=103)	*p* value
Follow-up duration (month)	29.72±27.52	32.59±33.82	0.373
Age (yr)	36.61±13.92	33.36±12.68	0.025
Male (%)	57.8	38.8	0.001
BMI‡ (kg/m²)	23.36±3.23	23.67±3.76	0.511
SBP§ (mmHg)	126.28±19.96	125.64±16.86	0.782
DBP‖ (mmHg)	79.77±13.21	79.5±11.63	0.863
Hypertension (%)	32	36.3	0.507
eGFR† (mL/min/1.73 m²)	78.19±33.33	74.89±31.21	0.375
eGFR <60 mL/min/1.73 m² (%)	28.9	31.1	0.715
Serum creatinine (mg/dL)	1.28±1.10	1.38±1.68	0.464
Spot urine PCR¶	1.97±2.91	1.98±3.48	0.984
Spot urine PCR¶ >3 (%)	17.1	13.1	0.412
Serum_albumin (mg/dL)	3.85±0.63	3.72±0.73	0.113
Serum total cholesterol (mg/dL)	191.37±63.27	210.55±99.38	0.093
Serum uric acid (mg/dL)	6.13±1.75	5.65±2.01	0.033
ACEI/ARB use (%)	64.4	68.8	0.467
Steroid use (%)	12	12.9	0.863

*Without T allele means A allele (male), AA (female) and the opposite is with T allele; †estimated Glomerular Filtration Rate; ‡Body Mass Index; §Systolic Blood Pressure; ‖Diastolic Blood Pressure; ¶Spot urine Protein Creatinine Ratio.

Table 4. Association between the presence of T allele and the prognosis of immunoglobulin A nephropathy

Variables	Allele	Stable disease	Progressive disease	*p* value
Total, N=480 (%)	Without T allele	334 (88.6)	43 (11.4)	0.024
	With T allele	99 (96.1)	4 (3.9)	
Male, N=258 (%)	Without T allele	186 (85.3)	32 (14.7)	0.127
	With T allele	38 (95)	2 (5)	
Female, N=222 (%)	Without T allele	148 (93.1)	11 (6.9)	0.359
	With T allele	61 (96.8)	2 (3.2)	

Survival analysis

With a univariate Cox proportional hazard model, the presence of T allele at AT2R A1818T, female gender, lower serum creatinine level at the time of kidney biopsy, absence of hypertension, and suPCR <3 were associated with better renal prognosis. With multivariate analysis, the presence of T allele and lower baseline serum creatinine level were independent factors associated with better prognosis (Table 5). The Kaplan-Meier survival curve in Fig. 1 shows that the survival rate of the patients with the patients with the T allele is significantly better than those without it. (Logrank test, *p*=0.0236).

DISCUSSION

Our study indicated that the A1818T polymorphism of AT2R gene is an independent prognostic factor for IgAN but not a significant susceptibility factor for IgAN development. To the best of our knowledge, this is the first report on the association between the A1818T polymorphism and the prognosis of primary renal disease. Various genetic factors have been reported to be associated with the susceptibility or prognosis of IgAN (17). Regarding chronic renal diseases,

Number at risk				
With T allele	15	7	3	2
Without T allele	49	15	5	2
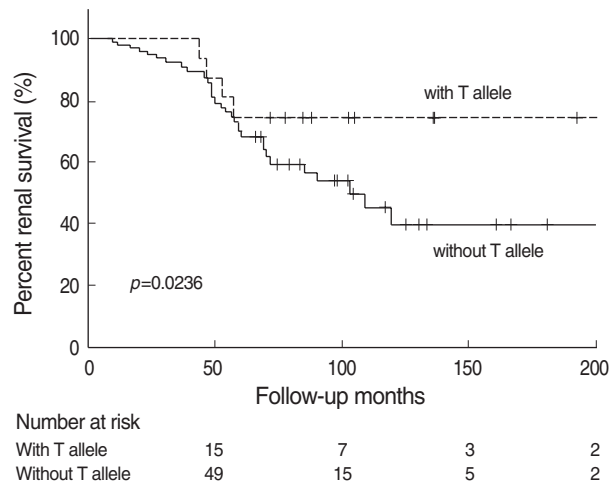
Fig. 1. Kaplan-Meier curve of survivorship of immunoglobulin A nephropathy according to allelic difference.

Table 5. Predictors for the disease progression of immunoglobulin A nephropathy by the Cox's proportional hazard model

Variables	Univariate analysis			Variables	Mutivariate analysis		
	p value	Exp (B)	95% CI		*p* value	Exp (B)	95% CI
With T allele*	0.025	0.309	0.111-0.862	With T allele*	0.041	0.221	0.052-0.940
Serum creatinine†	<0.001	1.277	1.171-1.394	Serum creatinine†	<0.001	1.901	1.504-2.402
Female gender	0.04	0.511	0.270-0.970				
Hypertension	0.043	1.966	1.020-3.790				
PCR >3	0.004	2.934	1.398-6.157				

*Change of AA allele to AT or TT allele in A1818T polymorphism; †per 1 mg/dL.

including IgAN and diabetic nephropathy, the genetic polymorphisms of genes involved in the renin-angiotensin system have been investigated most extensively because of their essential roles in the pathophysiology of chronic renal diseases and the renoprotective effects of RAS inhibitors on various chronic renal diseases, independent of blood pressure lowering effect. The counter-regulatory role of the AT2R in the biologic effects mediated by the ATR1 has raised a possible renoprotective role of AT2R and a novel therapeutic target for IgAN.

The variants of AT2R have been reported to be associated with various cardiovascular and renal diseases. AT2R gene null mice showed congenital anomalies of the kidney and urinary tract (18) and the AT2R gene transfer to the heart attenuated cardiac hypertrophy and fibrosis in a nongenetic rat model of hypertension (19). It has been reported that A to G transition in intron 1 of the AT2R gene was associated with congenital anomalies of kidney and urinary tract in Caucacians (18), but not in Japanese (16). The association of the AT2R gene variants with body mass index (20), the protective role of the AT2R gene in hypertensive subjects (21), and the association of AT2R gene polymorphism with the development of hypertension (22) and salt sensitivity (23) have been reported. It has been suggested that the +1675 G/A polymorphism of the AT2R gene is associated with early structural changes of the heart due to arterial hypertension (24). The variants of AT2R gene have been reported to be associated with a lower GFR and a higher pulse pressure in type 1 diabetes patients (14). Based on the results of this report, it is thought that the AT2R gene might be associated with clinical features of primary renal diseases.

Our study indicated that the T allele of the A1818T polymorphism of the AT2R gene was associated with better prognosis of IgAN in a relatively large number of patients. However, further functional studies on the biologic implications of polymorphism relevant to the pathophysiology of IgAN are needed to confirm our results. In addition, further genetic research with a large sample sized and a longer follow-up period is required to determine the interaction between conventional risk factors and various genetic risk factors.

In conclusion, the results of this study suggest that the A1818T polymorphism of the AT2R gene may be associated with the prognosis of IgAN, but not with susceptibility to IgAN and also, these results need to be confirmed in different ethnic groups.

APPENDIX

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