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Impact of the -675 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene on childhood IgA nephropathy

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Abstract. Plasminogen activator inhibitor-1 (PAI-1) is an important regulator of the fibrinolytic pathway and extracellular matrix (ECM) turnover. The -675 4G/5G polymorphism in the *PAI-1* promoter is associated with altered PAI-1 transcription, suggesting that this polymorphism may be a candidate risk factor for diseases characterized by ECM accumulation, such as immunoglobulin A nephropathy (IgAN) and mesangial proliferative glomerulonephritis (MesPGN). We genotyped childhood patients with biopsy-confirmed IgAN (n=111) and MesPGN (n=47), and healthy control subjects (n=230) for the -675 4G/5G PAI-1 polymorphism by polymerase chain reaction-restriction fragment length polymorphism methods. The distribution of the 4G/4G (27.9%), 4G/5G (45.1%) and 5G/5G (27.0%) genotypes in IgAN patients was significantly different from the healthy controls (32.2, 54.3 and 13.5%, respectively) (p=0.0092). There was no significant difference in the genotype distributions of the 4G/5G polymorphism between MesPGN patients and the healthy controls. Regarding the impact of the polymorphism on IgAN, the 4G/4G genotype was markedly increased in patients with proteinuria (≥1,000 mg/day) and/or hypertension when compared to patients without proteinuria and hypertension (OR=5.23, 95% CI 1.34-20.38, P=0.0183). These findings indicate that the *PAI-1* gene polymorphism may affect the susceptibility of childhood IgAN.

Introduction

Fibrinolytic activity is associated with excessive extracellular matrix (ECM) accumulation and is a predictor for glomerulonephritis (GN), which is related to ECM accumulation (1,2). The decreased fibrinolysis is mainly due to elevated plasminogen activator inhibitor-1 (PAI-1), which is the major inhibitor of both tissue-type and urokinase-type plasminogen activators (3).

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PAI-1 is a 50-kDa glycoprotein that belongs to the serine protease inhibitor superfamily (4,5). Elevated PAI-1 activity in plasma and glomerulus is observed during experimental and human GN (6-8). The human *PAI-1* gene has eight polymorphisms that have been described to date. The single-base pair insertion/deletion 4G/5G polymorphism at position -675 of the *PAI-1* promoter is of interest because of its association with altered PAI-1 transcription (9). This suggests that the 4G/5G polymorphism may be a candidate risk factor for diseases characterized by ECM accumulation, such as immunoglobulin A nephropathy (IgAN), mesangial proliferative glomerulonephritis (MesPGN), lupus nephritis and diabetic nephropathy. It has also been reported that the PAI-1 polymorphism is not associated with genesis of IgAN (10,11). On the contrary, it was reported that the 4G/5G polymorphism is associated with disease activity for primary membranous nephropathy (12), lupus nephritis and the development of type 2 diabetic nephropathy (13,14). However, the impact of the 4G/5G polymorphism on the disease activity and clinical course in childhood IgAN and MesPGN remains unknown.

IgAN and MesPGN are the most prevalent patterns among childhood-developed primary GN in the world (15-17); however there are striking geographic variations (18). Furthermore, the variable clinical courses from spontaneous remission to terminal renal failure even within the same histological entity have been shown (19), which implies that genetic factors may influence the progression as well as the development of these diseases.

In this study, we evaluated whether the 4G/5G polymorphism of the *PAI-1* gene is associated with a risk of childhood-developed IgAN and MesPGN in a relatively genetically homogeneous Korean population.

Materials and methods

Patients and control subjects. Patients diagnosed as IgAN and MesPGN between the years 1998 and 2002 at Kyung Hee University Hospital were retrospectively studied. Among those, childhood patients with follow-up duration >1 year and an onset age <20 years were enrolled in this study. Consecutively, participating subjects consisted of IgAN (n=111) and MesPGN (n=47) patients, and 230 healthy controls (mean age 55.64±13.64 years; range 30-76) attending the hospital for regular health check-ups (Table I). All diagnoses of IgAN and MesPGN were based on renal biopsy specimens using immunohistochemistry and electron microscopy. According

to their clinical outcome, patients were divided into subgroups: remission (negative >3 times in urine analysis) or persistence (positive >3 times in urine analysis) of hematuria; with (≥300 mg/day) or without (<300 mg/day) proteinuria; normal blood pressure or hypertension. To evaluate the impact of the *PAI-1* gene polymorphism on disease progression, patients were stratified into the remissor and progressor groups. Remissor meant patients who had a normal renal function and those who were without hematuria and proteinuria. Progressor meant patients who had decline of renal function or severe proteinuria (≥1,000 mg/day) and/or hypertension among those with persistent hematuria. This study was approved by the Ethics Review Committee of the Medical Research Institute at the Kyung Hee University Hospital in Seoul, Korea, and all participants provided informed consent.

DNA isolation and genotyping. DNA was isolated from venous blood using a genomic DNA purification kit (Nucleospin®, Düren, Germany). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)-based genotyping of the 4G/5G polymorphism was performed as described (20). Briefly, PCR was carried out in each sample containing genomic DNA from patients or controls with the following primers: 5'-TCCAACCTCAGCCAGACAAG-3' and 5'-TGATACACGGCTGACTCACC-3'. The amplification products for the 4G/5G polymorphism were digested with *Dra*III (New England Biolabs, Beverly, MA, USA) at 37˚C. The *PAI-1* gene has two alleles at position -675; 4G allele gave two fragments of 71 and 19 bp, and 5G allele gave a single 100-bp fragment. The accuracy of PCR-RFLP was confirmed three times by analysis and direct sequencing in several randomly selected samples.

Statistical analysis. The differences in observed genotypes and alleles between the patients and control subjects were compared by the Chi-square test. The correlation between polymorphism and clinical outcomes was analyzed using Chi-square test or Fisher's exact test. Two-sided P-values of <0.05 were considered significant. Odds ratio (OR) and 95% confidence intervals (CI) were calculated as estimates of the relative risks. The GraphPad PRISM statistical package (ver 2.00; Graphpad Software Inc., San Diego, CA, USA) was used.

Results

To determine whether the 4G/5G polymorphism is associated with IgAN and MesPGN, a total of 340 participants (230 controls, 111 IgAN and 47 MesPGN patients) were genotyped for the 4G/5G polymorphism. The distribution of the 4G/4G (27.9%), 4G/5G (45.1%) and 5G/5G (27.0%) genotypes in IgAN patients was significantly different from the healthy controls (32.2, 54.3 and 13.5%, respectively) (P=0.0092). The frequency of 4G (50.5%) and 5G (49.5%) allele in the patients also differed from the healthy controls (4G, 59.3% and 5G, 40.7%, P=0.0281). The association of the 4G/5G polymorphism with MesPGN was also evaluated by genotyping. No significant differences were detected in the genotype and allelic distributions between MesPGN patients and healthy controls (Table II).

To determine the related factors of IgAN, the associations between the 4G/5G polymorphism and clinical outcomes were assessed. Patients with proteinuria were associated with a higher carriage rate of the 4G allele when compared to patients without proteinuria (OR=2.78, 95% CI 1.13-6.82, P=0.0219). All hypertensive IgAN patients had the 4G allele, and the OR of 4G allele was higher than that of the normotensive patients. However, significance was not observed (OR=7.05, 95% CI 0.39-126.2, P=0.1051). There were no significant differences in the genotype distributions between remission and persistence of hematuria, over (>10 years) and below (≤10 years) the mean onset age, female and male patients, and other clinical outcomes (Table III). Finally, the impact of the *PAI-1* gene polymorphism on disease progression was assessed. The 4G/4G genotype was markedly increased in the progressor with proteinuria (≥1,000 mg/day) and/or hypertension group when compared to remissor without proteinuria and hypertension group (OR=5.23, 95% CI 1.34-20.38, P=0.0183) (Table IV).

Table I. Clinical characteristics of patients and controls.

Characteristics	Control (n=230)	IgAN (n=111)	MesPGN (n=47)
Onset age, years (range)	-	9.81±2.40 (2-16)	10.09±2.53 (2-19)
Gender (M:F)	125:105	55:56:00	22:25
Follow-up duration, months (range)	-	15.66±5.89 (12-67)	18.85±6.67 (12-61)
With proteinuria (≥300 mg/day)	0	36	16
Hypertension	0	8	7
Persistence of hematuria	0	72	26

IgAN, IgA nephropathy; MesPGN, mesangial proliferative glomerulonephritis.

Discussion

In this study, we presented the novel association of the 4G/5G polymorphism with a risk of childhood developed-IgAN in a relatively genetically homogeneous Korean population. The distributions of the 4G/5G allele in the control subjects were different from that in Caucasian populations (21,22). For example, the 4G allele frequency in the Korean population from this study was higher than that in the Caucasian population. However, the allelic frequency of this study is consistent with those reported in other Korean-based studies concerning the 4G/5G polymorphism (23), speculating that the difference could be due to racial variance.

The frequency of the 4G/4G genotype was reduced in IgAN, indicating a protective effect of the 4G/4G genotype for IgAN. This result is contradictory to previous reports that the 4G allele was associated with increased basal level of *PAI-1* gene transcription (24), and therefore the 4G/4G genotype was associated with increased plasma PAI-1 levels and the risk of GN (13,14). However, when IgAN patients were divided according to the presence of proteinuria and hypertension, the

Table II. Distribution of the 4G/5G polymorphism in patients and controls.

	Control	Total GN		IgAN		MesPGN	
	n (%)	n (%)	P-value[a]	n (%)	P-value[a]	n (%)	P-value[a]
Genotype							
4G/4G	74 (32.2)	48 (30.4)	0.0161	31 (27.9)	0.0092	17 (36.2)	0.4167
4G/5G	125 (54.3)	71 (44.9)		50 (45.1)		21 (44.7)	
5G/5G	31 (13.5)	39 (24.7)		30 (27.0)		9 (19.1)	
Allele							
4G	273 (59.3)	167 (52.8)	0.0726	112 (50.5)	0.0281	55 (58.5)	0.8804
5G	187 (40.7)	149 (47.2)		110 (49.5)		39 (41.5)	
Carriage rate							
4G (+)	199 (86.5)	119 (75.3)	0.0048	81 (73.0)	0.0022	38 (80.9)	0.3135
4G (-)	31 (13.5)	39 (24.7)		30 (27.0)		9 (19.1)	

[a]Chi-square test; patients vs. control subjects in each analysis. GN, glomerulonephritis; IgAN, IgA nephropathy; MesPGN, mesangial proliferative glomerulonephritis.

Table III. Association of the 4G allele carriage rate with clinical parameters.

Characteristics	n (carriage rate %)			
	Total GN		IgAN	
	4G (-)	4G (+)	4G (-)	4G (+)
With proteinuria	7 (13.5)	45 (86.5)[a]	5 (13.9)	31 (86.1)[c]
Without proteinuria	32 (30.2)	74 (69.8)	25 (33.3)	50 (66.7)
With hypertension	0 (0.0)	15 (100)[b]	0 (0.0)	8 (100)
Without hypertension	39 (27.3)	104 (72.7)	30 (29.1)	73 (70.9)
Persistence of hematuria	23 (23.5)	75 (76.5)	19 (26.4)	53 (73.6)
Remission of hematuria	16 (26.7)	44 (73.3)	11 (28.2)	28 (71.8)
<10 years of age	19 (24.7)	58 (73.3)	14 (25.0)	42 (75.0)
≥10 years of age	20 (24.7)	61 (75.3)	16 (29.1)	39 (70.9)
Female	19 (23.5)	62 (76.5)	13 (23.2)	43 (76.8)
Male	20 (26.0)	57 (74.0)	17 (30.9)	38 (69.1)

[a]Chi-square test, p=0.0219, OR=0.36, 95% CI 0.15-0.88; total GN patients with proteinuria are compared to patients without proteinuria. [b]Fisher's exact test, p=0.0230, OR=0.09, 95% CI 0.01-1.46; total GN patients with hypertension are compared to patients without hypertension. [c]Fisher's exact test, p=0.0397, OR=3.10, 95% CI 1.07-8.95; IgAN patients with proteinuria are compared to patients without proteinuria. GN, glomerulonephritis; IgAN, IgA nephropathy.

Table IV. Distribution of the 4G/5G polymorphism in remission and progression patients.

	Remissor, n (%)	Progressor, n (%)	OR (95% CI)	P-value[a]
Genotype				
4G/4G	4 (11.4)	8 (38.1)	5.23 (1.34-20.38)	0.0183
4G/5G	22 (54.3)	9 (42.9)	0.55 (0.19-1.60)	0.2681
5G/5G	12 (34.3)	4 (10.0)	0.51 (0.14-1.85)	0.3702
Alleles				
4G	30 (39.5)	25 (59.5)	2.26 (1.05-4.87)	0.0366
5G	46 (60.5)	17 (40.5)	0.44 (0.21-0.96)	

[a]Chi-square test or Fisher's exact test; progressor vs. remissor in each analysis. OR, odds ratio; CI, confidence intervals; Remissor, patients with normal renal function and without hematuria and proteinuria; Progressor, patients with persistent hematuria and severe proteinuria (≥1,000 mg/day) and/or hypertension.

4G/4G genotype and 4G allele were increased in those with proteinuria and/or hypertension. The 4G/4G genotype and 4G allele in MesPGN patients with proteinuria and hypertension also were increased (data not shown), although there was no significance. Furthermore, recent studies also indicate that the 4G/4G genotype was increased in type 2 diabetic nephropathy with hypertension and in lupus nephritis patients with heavier proteinuria (13,14). These results suggest that the 4G/4G genotype may be closely associated with disease activity of GN, including IgAN, diabetic nephropathy and lupus nephritis.

The impact of the 4G/5G polymorphism on progression of IgAN was assessed. In this study, IgAN patients with renal impairment were not observed during follow-up duration. We thus evaluated the association between 4G/5G and other poor prognostic factor (16), such as ≥1,000 mg/day proteinuria and hypertension. The remission rate of IgAN was approximately 34% and the possible progression rate was approximately 19%, which partially consisted with the long-term outcomes of IgAN (25). The frequency of the 4G/4G genotype in the progressor group was 5-fold higher than that in the remissor group. These results suggest that the 4G/4G genotype may affect the clinical course of remission or progression in childhood IgAN, although further long-term studies are required.

In conclusion, the 4G/5G polymorphism of the *PAI-1* gene is associated with the susceptibility to IgAN, and the 4G/4G genotype may affect the disease activity and clinical course of childhood IgAN.

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