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## Original Article

# Impact of gene polymorphisms of interleukin-18, transforming growth factor- $\beta$ , and vascular endothelial growth factor on development of IgA nephropathy and thin glomerular basement membrane disease



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## ABSTRACT

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**Background:** We investigated the effects of gene polymorphisms on the development of IgA nephropathy and thin glomerular basement membrane (GBM) disease by analyzing polymorphisms in the interleukin (IL)-18, transforming growth factor (TGF)- $\beta$ , and vascular endothelial growth factor (VEGF) genes in Korean patients.

**Methods:** This study included 146 normal individuals and 69 biopsy-proven IgA nephropathy and 44 thin GBM disease patients. The gene polymorphisms – 607 A/C and – 137 G/C in IL-18, – 509C/T and T869C in TGF- $\beta$ , and – 2578C/A and 405C/G in VEGF were investigated in DNA extracted from peripheral blood.

**Results:** The frequencies of the IL-18 – 607CC genotype (43.5% vs. 21.2%,  $P=0.002$ ,  $P_{corrected}=0.012$ ) and the VEGF 405 GG genotype (37.7% vs. 21.2%,  $P=0.002$ ,  $P_{corrected}=0.012$ ) were significantly increased in the IgA nephropathy group compared with the control group, whereas no significant differences in genotype frequency were observed between the thin GBM disease and control groups. However, there were no significant differences in genotype and allele frequencies between the IgA nephropathy and thin GBM disease groups.

**Conclusion:** This study did not show any statistically significant differences of six selected gene polymorphisms of the IL-18, TGF- $\beta$ , and VEGF genes between IgA nephropathy and thin GBM disease. Additional extensive studies are required to clarify the potential role of gene polymorphism to discriminate IgA nephropathy and thin GBM disease without renal biopsy.

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## Introduction

IgA nephropathy and thin glomerular basement membrane (GBM) disease are representative glomerular diseases that cause asymptomatic hematuria [1]. Clinical progress of IgA nephropathy takes diverse forms ranging from reaching the clinical remission stage without treatment to the development of the terminal stage of renal failure. It was

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reported that approximately 30–50% of the IgA nephropathy patients reached the final stage of renal failure within 30 years [2]. Some of the patients with thin GBM disease were reported to have developed renal failure [3], but the prognosis is generally favorable [4]. Therefore, differential diagnosis of IgA nephropathy and thin GBM disease is an important part of determining the treatment method in patients with asymptomatic hematuria.

IgA nephropathy and thin GBM disease are generally diagnosed by renal biopsy. A characteristic of IgA nephropathy is that IgA deposition can be seen in the mesangium area in immunofluorescence microscopy. Deposition of electro-dense material in the mesangium or paramesangium areas can also be seen by electron microscopy [5]. The thickness of the glomerular basement membrane measured by electron microscopy, the criterion for the histological diagnosis of thin GBM disease, is known to be below 200–250 nm in children and below 250–264 nm in adults [6] depending on the age. Thin GBM disease is diagnosed when no deposition of immunoglobulin or complement is shown in immunofluorescence microscopy and there is a general decrease in the thickness of the GBM in at least 50% of the capillary loop [4].

The pathogenesis of IgA nephropathy is not clearly known yet. There are a number of studies that were based on the assumption that cytokines, control hormones produced during the active and effective phases of immunological reactions that transmit signals between cells [7], have a certain role in IgA nephropathy, but these reports have diverse results [8,9]. Although the pathogenesis of thin GBM disease is not completely known yet, genetic abnormalities are suspected since there is a high tendency for familial association [4].

It has been reported in many studies that the genetic polymorphisms of interleukin (IL)-18, transforming growth factor (TGF)- $\beta$ , and vascular endothelial growth factor (VEGF) were involved in the development and progression of a variety of glomerular diseases including IgA nephropathy [10–16]. However, cytokines are considered to have less effect on the development of thin GBM disease from a pathogenic point of view and there has been no study on cytokine gene polymorphisms. This study investigated the impact of different genetic polymorphisms on the development of IgA nephropathy and thin GBM disease and attempted to evaluate the potential of this approach for differential diagnosis

between the two diseases by conducting the test for the genetic polymorphisms of IL-18, TGF- $\beta$ , and VEGF in IgA nephropathy and thin GBM disease patients and normal individuals.

## Methods

### Patients

This was a case-control, cross-sectional study, performed in a single center. A total of 113 Korean patients among IgA nephropathy and thin GBM disease patients who underwent renal biopsy at the Kyungpook National University Hospital (Daegu, Korea) agreed to genotyping analysis and were recruited. The control group consisted of 146 unrelated, healthy Korean individuals with normal renal function, normotension, no known medical problems based on a health-screening questionnaire, and who agreed to genotyping analysis. The controls were enrolled from the health promotion center of the same institute as the patients. The present study was approved by the institutional review board of Kyungpook National University Hospital (KNUH 2011-04-010).

### Genomic DNA extraction

Blood samples (3 mL) were collected in ethylenediaminetetraacetic acid tubes, and genomic DNA was isolated from 200  $\mu$ L of whole blood using the Fujifilm DNA whole blood kit S with the QuickGene-810 system (Fujifilm, Tokyo, Japan) according to the manufacturer's instructions. The extracted DNA was assessed for purity, yield, and concentration on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Purity was monitored by the A260/280 ratio. The DNA was diluted to 10 ng/ $\mu$ L for a working solution, and the isolated DNA was stored at  $-20^{\circ}\text{C}$ .

### Cytokine gene polymorphism typing

Polymerase chain reaction (PCR) and melting curve analyses were performed on a LightCycler480 (Roche Diagnostics, Penzberg, Germany). The primers and hybridization probes used in this study were designed using available reference

**Table 1. Primer and hybridization probe sequences**

Primer	Sequence (5' → 3')	Probe	Size (bp)	Annealing temp (°C)
IL-18 -607 A/C	F: GTTGATACAGGCCATTAAGATT R: CCCTAAATATATGTATCCTTAAATTG	GAAAGTGTAAAAATTATTACATAAAATTCT- FL LCred640-ATGATGGTATCCGTGTGGCTTG-p	295	49
-137 G/C	F: AGTGGCAGAGGATACGAG R: AAGAGATACTCAGAAAGAGGTACA	TCATGAAATCTTTTCTCCGTAAAAGT- FL LCred640-GGGCTCTGTGCCTTCCAAA-p	121	52
TGF- $\beta$ -509C/T	F: GTAAATGGGGACAGTAAATGTATG R: CTGGGAAACAAGGTAGGAGAA	TCCATCCCTCAGGTGTCC- FL LCred640-GTGCCCTCTCCCACTGA-p	216	54
869 T/C	F: ATCTAGGTTATTTCCGTGGGATAC R: CAGCTCATGTGCATAGTCTTG	CTGCTGCCGCTGCTGC FL LCred640-CCGCTGTGTGGCTACTGGTGCTGAC-p	326	54
VEGF -2578C/A	F: GAGGCTATGTACAGCTGTAGGTCA R: CCTCCAATCTCCACATCTTC	ACCCTGGCAGATCTGG- FL LC640-GGATAATCAGACTGACTGGTCCCACTCTTC-p	225	66
405C/G	F: TTGCCATTCCCACTTGAA R: CCGGGGAGGAGGTGGTA	CGGTCACCCCAAAAAGCAGGTAC- FL LCred640-CACTTTGCCCTGTCCCTTTCG-p	327	68

F, forward primer; FL, fluorescence; IL-18, interleukin-18; p, phosphorylation; R, reverse primer; TGF- $\beta$ , transforming growth factor- $\beta$ ; VEGF, vascular endothelial growth factor.

**Table 2. Demographic characteristics of IgA nephropathy, thin GBM disease, and normal controls**

	IgA (n=69)	Thin GBM (n=44)	Normal (n=146)	P
Age (y)	34.22 ± 13.50	33.66 ± 14.95	42.40 ± 11.73	< 0.001
Sex (male:female)	42:27	24:20	29:117	< 0.001
Blood urea nitrogen (mg/dl)	14.98 ± 5.70	12.38 ± 3.11		0.006
Serum creatinine (mg/dl)	0.92 ± 0.43	0.73 ± 0.16		0.005
Estimated GFR	107.78 ± 42.68	125.46 ± 37.12		0.026
24-h proteinuria (mg/d)	1059.48 ± 1580.17	272.45 ± 472.12		0.002

Values are shown as mean ± standard deviation.

GBM, glomerular basement membrane; GFR, glomerular filtration rate (calculated using Modification of Diet in Renal Disease formula).

**Table 3. Cytokine genotype distributions (%) and allele frequencies in the IgA nephropathy and control groups**

IL-18 -607A→C polymorphism							
	A/A	A/C	C/C	P*	A allele	C allele	P†
IgA (n=69)	10 (14.5)	29 (42.0)	30 (43.5)		0.355	0.645	
Control (n=146)	40 (27.4)	75 (51.4)	31 (21.2)	0.002 (0.012)	0.531	0.469	0.001 (0.006)
Dominant effect (AA vs. AC+CC)				0.037 (0.222)	OR 2.226, CI 1.039–4.773		
Recessive effect (AA+AC vs. CC)				0.001 (0.006)	OR 2.854, CI 1.536–5.302		
IL-18 -137G→C polymorphism							
	G/G	G/C	C/C	P*	G allele	C allele	P†
IgA (n=69)	53(76.8)	16 (23.2)	0 (0.0)		0.116	0.884	
Control (n=146)	111 (76.0)	30 (20.5)	5 (3.4)	0.352	0.863	0.137	0.545
Dominant effect (GG vs. GC+CC)				0.900	OR 1.044, CI 0.531–2.053		
Recessive effect (GG+GC vs. CC)				0.179	OR 0.966, CI 0.937–0.996		
TGF-β -509C→T polymorphism							
	C/C	C/T	T/T	P*	C allele	T allele	P†
IgA (n=69)	14 (20.3)	35(50.7)	20 (29.0)		0.457	0.543	
Control (n=146)	43 (29.5)	63 (43.2)	40 (27.4)	0.347	0.510	0.490	0.298
Dominant effect (CC vs. CT+TT)				0.155	OR 1.640, CI 0.826–3.257		
Recessive effect (CC+CT vs. TT)				0.808	OR 1.082, CI 0.573–2.040		
TGF-β 869T→C polymorphism							
	T/T	T/C	C/C	P*	T allele	C allele	P†
IgA (n=69)	12 (17.4)	36 (52.2)	21 (30.4)		0.565	0.435	
Control (n=146)	43 (29.5)	63 (43.2)	40 (27.4)	0.162	0.510	0.490	0.144
Dominant effect (TT vs. TC+CC)				0.057	OR 0.504, CI 0.246–1.033		
Recessive effect (TT+TC vs. CC)				0.645	OR 0.863, CI 0.460–1.617		
VEGF -2578C→A polymorphism							
	C/C	C/A	A/A	P*	C allele	A allele	P†
IgA (n=69)	42 (60.9)	24 (34.8)	3 (4.3)		0.783	0.217	
Control (n=146)	82 (56.2)	56 (38.4)	8 (5.5)	0.795	0.753	0.247	0.296
Dominant effect (CC vs. CA+AA)				0.514	OR 1.214, CI 0.677–2.176		
Recessive effect (CC+CA vs. AA)				1.000	OR 1.275, CI 0.328–4.964		
VEGF 405C→G polymorphism							
	C/C	C/G	G/G	P*	C allele	G allele	P†
IgA (n=69)	11 (15.9)	32 (46.4)	26 (37.7)		0.391	0.609	
Control (n=146)	41 (28.1)	74 (50.7)	31 (21.2)	0.002 (0.012)	0.534	0.466	0.006 (0.036)
Dominant effect (CC vs. CG+GG)				0.555	OR 1.188, CI 0.670–2.109		
Recessive effect (CC+CG vs. GG)				0.001 (0.006)	OR 2.243, CI 1.197–4.203		

\* Prefers to genotype distribution.

† Prefers to allele frequency; data were analyzed using the Chi-square test or Fisher's exact test as appropriate. P-values were Bonferroni corrected (blank) for multiple testing.

CI, 95% confidence interval; IL-18, interleukin-18; OR, odds ratio TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

sequences from the GenBank database by TIB-MOLBIOL (Berlin, Germany; Table 1). Real-time PCR was performed as previously described [11].

### Statistical analysis

The results are expressed as the mean  $\pm$  standard deviation or frequencies. The Hardy-Weinberg equilibrium for each polymorphism was tested using the Chi-square test. The differences in allele frequency and genotype distribution between the two groups were assessed by Chi-square analysis. The odds ratio (OR) and 95% confidence interval (CI) were calculated. Logistic regression analysis was used to adjust for significantly different clinical variables. Statistical analyses were performed using SPSS

18.0 for Windows (SPSS Inc., Chicago, IL, USA). A  $P$  value  $< 0.05$  was considered significant. When multiple comparisons were involved, corrected  $P$  values were calculated for multiple testing by the Bonferroni method.

### Results

This study included 146 normal subjects and 69 IgA nephropathy and 44 thin GBM disease patients. The mean age and the sex ratio were significantly different among the groups ( $P < 0.001$  and  $P < 0.001$ , respectively). Significant differences between the IgA nephropathy and thin GBM disease groups were observed in all of the serum urea nitrogen ( $15.0 \pm 5.7$  mg/dL vs.  $12.4 \pm 3.1$  mg/dL,  $P = 0.006$ ), serum creatinine ( $0.92 \pm 0.43$  mg/dL vs.

**Table 4. Cytokine genotype distributions (%) and allele frequencies in the IgA nephropathy and control groups after age- and sex-adjusted logistic regression analysis**

	IgA nephropathy (n=69)	Control group (n=146)	$P^*$	Odds ratio	95% CI
<b>IL-18 -607 A/C</b>					
Genotype					
AA	10 (14.5%)	40 (27.4)		1	
AC	29 (42.0%)	75 (51.4)	0.387	1.491	0.604–3.684
CC	30 (43.5%)	31 (21.2)	0.008 (0.048)	3.693	1.400–9.740
Allele					
A	49 (35.5)	155 (53.1)		1	
C	89 (64.5)	137 (46.9)	0.005 (0.030)	1.993	1.237–3.209
<b>IL-18-137 G/C</b>					
Genotype					
GG	53 (76.8)	111 (76.0)		1	
GC	16 (23.2)	30 (20.5)	0.999		
CC	0 (0.0)	5 (3.4)	0.357	1.460	0.653–3.266
Allele					
G	122 (88.4)	252 (86.3)		1	
C	16 (11.6)	40 (13.7)	0.683	0.865	0.432–1.733
<b>TGF-<math>\beta</math> -509 C/T</b>					
Genotype					
CC	14 (20.3)	43 (29.5)		1	
CT	35 (50.7)	63 (43.2)	0.081	2.108	0.912–4.871
TT	20 (29.0)	40 (27.4)	0.172	1.900	0.756–4.773
Allele					
C	63 (45.7)	149 (51.0)		1	
T	75 (54.3)	143 (49.0)	0.167	1.386	0.872–2.204
<b>TGF-<math>\beta</math> 869 T/C</b>					
Genotype					
TT	12 (17.4)	43 (29.5)		1	
TC	36 (52.2)	63 (43.2)	0.070	2.393	0.933–6.141
CC	21 (30.4)	40 (27.4)	0.040	2.476	1.040–5.891
Allele					
T	60 (43.5)	149 (51.0)		1	
C	78 (56.5)	143 (49.0)	0.070	1.538	0.966–2.451
<b>VEGF -2578 C/A</b>					
Genotype					
CC	42 (60.9)	82 (56.2)		1	
CA	24 (34.8)	56 (38.4)	0.940	1.059	0.243–4.619
AA	3 (4.3)	8 (5.5)	0.590	0.827	0.414–1.650
Allele					
C	108 (78.3)	220 (75.3)		1	
A	30 (21.7)	72 (24.7)	0.746	0.914	0.529–1.578
<b>VEGF 405C/G</b>					
Genotype					
CC	11 (15.9)	41 (28.1)		1	
CG	32 (46.4)	74 (50.7)	0.226	1.727	0.713–4.183
GG	26 (37.7)	31 (21.2)	0.010 (0.060)	3.613	1.362–9.585
Allele					
C	54 (39.1)	156 (53.4)		1	
G	84 (60.9)	136 (46.6)	0.008 (0.048)	1.885	1.179–3.015

\*  $P$ -values were Bonferroni corrected (blank) for multiple testing.

CI, confidence interval; IL-18, interleukin-18; TGF- $\beta$ , transforming growth factor- $\beta$ ; VEGF, vascular endothelial growth factor.

0.73 ± 0.16 mg/dL,  $P=0.005$ ), glomerular filtration rate (107.8 ± 42.7 mL/min/1.73 m<sup>2</sup> vs. 125.5 ± 37.1 mL/min/1.73 m<sup>2</sup>,  $P=0.026$ ), and 24-hour proteinuria (1059.5 ± 1580.2 mg/day vs. 272.5 ± 472.1 mg/day,  $P=0.002$ ; Table 2). The genotype frequencies of

the patient and control groups did not deviate significantly from the Hardy-Weinberg equilibrium ( $P > 0.05$ ).

The frequencies of the IL-18 -607CC genotype showed a significant difference between the IgA nephropathy group and

**Table 5. Cytokine genotype distributions (%) and allele frequencies in the thin GBM disease and control groups**

IL-18 -607A→C polymorphism							
	A/A	A/C	C/C	$P^*$	A allele	C allele	$P^\dagger$
Thin GBM (n=44)	7 (15.9)	23(52.3)	14(31.8)		0.420	0.580	
Control (n=146)	40 (27.4)	75 (51.4)	31 (21.2)	0.182	0.531	0.469	0.069
Dominant effect (AA vs. AC+CC)				0.122	OR 1.995, CI 0.822–4.837		
Recessive effect (AA+AC vs. CC)				0.148	OR 0.578, CI 0.273–1.221		
IL-18 -137G→C polymorphism							
	G/G	G/C	C/C	$P^*$	G allele	C allele	$P^\dagger$
Thin GBM (n=44)	32(72.7)	11 (25.0)	1 (2.3)		0.852	0.148	
Control (n=146)	111 (76.0)	30 (20.5)	5 (3.4)	0.826	0.863	0.137	0.799
Dominant effect (GG vs. GC+CC)				0.657	OR 1.189, CI 0.554–2.555		
Recessive effect (GG+GC vs. CC)				0.702	OR 1.525, CI 0.173–13.409		
TGF-β -509C→T polymorphism							
	C/C	C/T	T/T	$P^*$	C allele	T allele	$P^\dagger$
Thin GBM (n=44)	12 (27.3)	21 (47.7)	11 (25.0)		0.511	0.489	
Control (n=146)	43 (29.5)	63 (43.2)	40 (27.4)	0.866	0.510	0.490	0.986
Dominant effect (CC vs. CT+TT)				0.780	OR 1.113, CI 0.524–2.364		
Recessive effect (CC+CT vs. TT)				0.753	OR 1.132, CI 0.522–2.453		
TGF-β 869T→C polymorphism							
	T/T	T/C	C/C	$P^*$	T allele	C allele	$P^\dagger$
Thin GBM (n=44)	12 (27.3)	21 (47.7)	11 (25.0)		0.511	0.489	
Control (n=146)	43 (29.5)	63 (43.2)	40 (27.4)	0.866	0.510	0.490	0.986
Dominant effect (TT vs. TC+CC)				0.780	OR 1.113, CI 0.524–2.364		
Recessive effect (TT+TC vs. CC)				0.753	OR 1.132, CI 0.522–2.453		
VEGF -2578C→A polymorphism							
	C/C	C/A	A/A	$P^*$	C allele	A allele	$P^\dagger$
Thin GBM (n=44)	26 (59.1)	17 (38.6)	1 (2.3)		0.784	0.216	
Control (n=146)	82 (56.2)	56 (38.4)	8 (5.5)	0.675	0.753	0.247	.555
Dominant effect (CC vs. CA+AA)				0.731	OR 0.887, CI 0.448–1.758		
Recessive effect (CC+CA vs. AA)				0.687	OR 2.493, CI 0.303–20.496		
VEGF 405C→G polymorphism							
	C/C	C/G	G/G	$P^*$	C allele	G allele	$P^\dagger$
Thin GBM (n=44)	5 (11.4)	22(50.0)	17 (38.6)		0.364	0.636	
Control (n=146)	41 (28.1)	74 (50.7)	31 (21.2)	0.019 (0.114)	0.534	0.466	0.005 (0.030)
Dominant effect (CC vs. CG+GG)				0.023 (0.138)	OR 3.046, CI 1.122–8.267		
Recessive effect (CC+CG vs. GG)				0.020 (0.120)	OR 0.428, CI 0.207–0.884		

\*  $P$  refers to genotype distribution;

†  $P$  refers to allele frequency; data were analyzed using the chi-square test or Fisher's exact test as appropriate;  $P$ -values were Bonferroni corrected (blank) for multiple testing.

CI, 95% confidence interval; GBM, glomerular basement membrane; IL-18, interleukin-18; OR, odds ratio; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.

control group (43.5% vs. 21.2%,  $P=0.002$ ,  $P$  corrected=0.012). The frequency of the -607A/C single nucleotide polymorphism (SNP) allele in IL-18 was investigated and A:C allele frequency ratio was 35.5:64.5 in the IgA nephropathy group and 53.1:46.9 in the control group showing a significant difference

between the two groups ( $P=0.001$ ,  $P$  corrected=0.006). In the recessive genetic analysis, the CC genotype was significantly more frequent in the IgA nephropathy group compared to the control group (43.5% vs. 21.2%,  $P=0.001$ ,  $P$  corrected=0.006, OR=2.854, 95% CI 1.536–5.302; Table 3). The frequencies of

**Table 6. Cytokine genotype distributions (%) and allele frequencies in the IgA nephropathy and thin GBM groups**

IL-18 -607 A→C polymorphism							
	A/A	A/C	C/C	$P^*$	A allele	C allele	$P^\dagger$
IgA (n=69)	10 (14.5)	29 (42.0)	30 (43.5)		0.355	0.645	
Thin GBM (n=44)	7 (15.9)	23(52.3)	14 (31.8)	0.452	0.420	0.580	.324
Dominant effect (AA vs. AC+CC)				0.837			
Recessive effect (AA+AC vs. CC)				0.215		OR 0.896, CI 0.314–2.599 OR 1.648, CI 0.746–3.643	
IL-18 -137 G→C polymorphism							
	G/G	G/C	C/C	$P^*$	G allele	C allele	$P^\dagger$
IgA (n=69)	53(76.8)	16 (23.2)	0 (0.0)		0.116	0.884	
Thin GBM (n=44)	32(72.7)	11 (25.0)	1 (2.3)	0.435	0.852	0.148	0.486
Dominant effect (GG vs. GC+CC)				0.624			OR 1.242, CI 0.522–2.958
Recessive effect (GG+GC vs. CC)				0.028		OR 1.023, CI 0.978–1.070	
TGF-β -509C→T polymorphism							
	C/C	C/T	T/T	$P^*$	C allele	T allele	$P^\dagger$
IgA (n=69)	14 (20.3)	35(50.7)	20 (29.0)		0.457	0.543	
Thin GBM (n=44)	12 (27.3)	21 (47.7)	11 (25.0)	0.679	0.511	0.489	0.421
Dominant effect (CC vs. CT+TT)				0.390			OR 0.679, CI 0.280–1.646
Recessive effect (CC+CT vs. TT)				0.643		OR 1.224, CI 0.519–2.888	
TGF-β 869 T→C polymorphism							
	T/T	T/C	C/C	$P^*$	T allele	C allele	$P^\dagger$
IgA (n=69)	12 (17.4)	36 (52.2)	21 (30.4)		0.565	0.435	
Thin GBM (n=44)	12 (27.3)	21 (47.7)	11 (25.0)	0.445	0.511	0.489	0.260
Dominant effect (TT vs. TC+CC)				0.210			OR 0.561, CI 0.226–1.394
Recessive effect (TT+TC vs. CC)				0.532		OR 1.313, CI 0.559–3.082	
VEGF -2578C→A polymorphism							
	C/C	C/A	A/A	$P^*$	C allele	A allele	$P^\dagger$
IgA (n=69)	42 (60.9)	24 (34.8)	3 (4.3)		0.783	0.217	
Thin GBM (n=44)	26 (59.1)	17 (38.6)	1 (2.3)	0.445	0.784	0.216	0.979
Dominant effect (CC vs. CA+AA)				0.210			OR 1.077, CI 0.498–2.329
Recessive effect (CC+CA vs. AA)				0.532		OR 3.359, CI 0.379–29.674	
VEGF 405C→G polymorphism							
	C/C	C/G	G/G	$P^*$	C allele	G allele	$P^\dagger$
IgA (n=69)	11 (15.9)	32 (46.4)	26 (37.7)		0.391	0.609	
Thin GBM (n=44)	5 (11.4)	22(50.0)	17 (38.6)	0.798	0.364	0.636	0.670
Dominant effect (CC vs. CG+GG)				0.496			OR 1.479, CI 0.477–4.590
Recessive effect (CC+CG vs. GG)				0.919		OR 0.963, CI 0.441–2.091	

\*  $P$  refers to genotype distribution;

†  $P$  refers to allele frequency; data were analyzed using the Chi-square test or Fisher's exact test as appropriate.

CI, 95% confidence interval; GBM, glomerular basement membrane; IL-18, interleukin-18; OR, odds ratio; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor.



the VEGF 405GG genotype were significantly increased in the IgA nephropathy group compared with the control group (37.7% vs. 21.2%,  $P=0.002$ ,  $P$  corrected=0.012). Investigation of the allele frequency of the 405C/G SNP in VEGF showed the allele ratio of C:G as 39.1:60.9 in the IgA nephropathy group and 53.4:46.6 in the control group showing a significant difference between the two groups ( $P=0.006$ ,  $P$  corrected=0.036). In the recessive genetic analysis, the GG genotype was significantly more frequent in the IgA nephropathy group compared to the control group (37.7% vs. 21.2%,  $P=0.001$ ,  $P$  corrected=0.006, OR=2.243, 95% CI 1.197–4.203). There were no significant differences in the genotype and allele distributions of the investigated cytokine gene polymorphisms other than the IL-18 –607A/C and VEGF 405C/G (Table 3). Comparative analysis between the IgA nephropathy group and control group using age- and sex-adjusted logistic regression analysis showed a significant difference in the genotype and allele distribution of the –607A/C SNP in IL-18 and in the allele frequency of the 405C/G SNP in VEGF (Table 4).

The genotype distribution of the 405C/G SNP in VEGF between the thin GBM disease group and control group showed a significant difference ( $P=0.019$ ). However, the difference became insignificant after correcting for multiple comparisons ( $P$  corrected=0.114). Significant differences could not be observed in the analysis of the dominant inheritance and regressive inheritance of the 405C/G SNP in VEGF. Investigation of the allele frequency of the 405C/G SNP in VEGF showed the allele ratio C:G as 36.4:63.6 in the thin GBM disease group and 53.4:46.6 in the control group, showing a significant difference between the two groups ( $P=0.005$ ,  $P$  corrected=0.030, Table 5). However, after age- and sex-adjusted comparative analysis between the thin GBM disease group and control group using logistic regression analysis, the genotype distribution and allele frequency of the 405C/G SNP in VEGF did not show a significant difference ( $P$  corrected=0.126 and 0.114, respectively). There were no significant differences in the genotype and allele distributions of the investigated cytokine gene polymorphisms other than VEGF 405C/G.

In the comparison between the IgA nephropathy group and thin GBM disease group, the genotype distribution and allele frequency were not significantly different and age- and sex-adjusted results did not show any significant differences (Table 6). When the IgA nephropathy patients were compared with the thin GBM disease patients according to the level of proteinuria (< 1.0 g/day vs.  $\geq$  1.0 g/day) or the glomerular filtration rate (< 60 mL/min vs.  $\geq$  60 mL/min), no significant differences were observed in the genotype distributions of the investigated cytokine gene polymorphisms (data not shown).

## Discussion

This study investigated the possible association of IL-18, TGF- $\beta$ , and VEGF gene polymorphisms with the development of IgA nephropathy and thin GBM disease. The polymorphism in the IL-18 promoter at position –607 is associated with the development of IgA nephropathy, and the C allele at IL-18 –607A/C and the G allele at VEGF 405C/G seem to be associated with the development of IgA nephropathy and may be risk alleles. However, no significant difference in polymorphisms could be observed between the thin GBM disease group and control group. Comparison between the IgA nephropathy

group and thin GBM disease group showed no significant difference in genotype distribution.

In a previous study, the associations of serum or urinary IL-18 levels with the development of glomerulonephritis have been reported [17,18]. These findings indicate that IL-18 levels are associated with the activity and development of idiopathic nephrotic syndrome. Among the IL-18 polymorphisms, homozygous for C at position –607 and G at position –137 had higher levels of IL-18 mRNA compared to other genotypes [10]. Although we did not measure the serum or urine IL-18 levels in the present study, the IL-18 –607CC genotype, which was related with higher levels of IL-18 mRNA, was significantly more frequent in the IgA nephropathy group. This finding is in concordance with previous studies that found that a high IL-18 level was associated with the development of glomerulonephritis [17,18].

The VEGF levels have been reported to be associated with the development and progression of glomerulonephritis [19]. The urinary VEGF level was correlated with baseline serum creatinine and erythrocyte sedimentation rate in glomerulonephritis patients. VEGF mRNA expression in peripheral blood mononuclear cells decreased in patients undergoing remission. VEGF expression analyses showed that the GG genotype at the 405 position of the VEGF promote region produced higher VEGF transcripts compared to the CC genotype [20]. Therefore, the VEGF 405GG genotype, which was significantly increased in the IgA patients in our study, might be associated with an increased risk of developing IgA nephropathy by producing higher VEGF transcripts than the other genotypes.

In our previous study, IL-18 and VEGF polymorphisms were associated with development of primary glomerulonephritis. In this study, 69% of glomerulonephritis was IgA nephropathy and the IL-18 –607CC genotype and VEGF 405GG genotype were associated with susceptibility to and the development of primary glomerulonephritis [11]. These data might reflect some associations between IgA nephropathy and IL-18 and VEGF polymorphisms.

However, our results could not further support that IL-18 and VEGF may be related to the progression of IgA nephropathy because no significant relationships were observed between the genotype distributions of IL-18 or VEGF, and the level of proteinuria or the glomerular filtration rate. Additional studies measuring serum and urine levels of IL-18 and VEGF and comparing histological grades are necessary to further clarify the potential roles of IL-18 and VEGF gene polymorphisms in the progression of IgA nephropathy.

The association of TGF- $\beta$  –509CC and 869CC gene polymorphisms with IgA nephropathy has been reported in several studies [12–15]. Taken together with the present study, TGF- $\beta$  gene polymorphisms have been reported to be associated with the susceptibility of IgA nephropathy in some races, but there is still weak evidence to prove the association of TGF- $\beta$  SNPs with IgA nephropathy in Asian populations. It is necessary to perform further extensive studies in various races to investigate the contribution of TGF- $\beta$  SNPs to the susceptibility of IgA nephropathy.

The pathogenesis of thin GBM disease is known to be associated with an abnormality in type 4 collagen synthesis and many COL4A3 and COL4A4 mutations have been reported [21]. These mutations could cause a glycine substitution inhibiting the formation of the collagen trimer. We hypothesized that the genetic polymorphisms affecting cytokine expression would have less effect on the development of thin GBM disease

than on IgA nephropathy. Comparing between the thin GBM disease and control groups, no significant difference could be observed in the genotype distributions and allele frequencies. This suggests that the genetic polymorphisms are not associated with the development of thin GBM disease. However, there was also no significant difference when the thin GBM disease group and IgA nephropathy group were compared. Since this may be due to the small number of thin GBM patients, further study is necessary that includes larger numbers of participants and measures cytokine concentrations in the serum or tissue.

Our study did have some limitations. First, the sample size of the phenotype groups was relatively small. Additional extensive studies are required to investigate the potential roles of cytokine gene polymorphisms to discriminate IgA nephropathy and thin GBM disease without renal biopsy. Second, we could not measure the serum or urine cytokine levels at the time of biopsy and gene expression of cytokines in peripheral blood mononuclear cells because of the cross-sectional study design. Further study comparing cytokine gene polymorphisms and measuring cytokine concentrations in serum or tissue might help to differentiate these two disease groups.

In the present study, the -607A/C IL-18 and 405C/G VEGF polymorphic variants, who might express higher levels of serum IL-18 and VEGF, had significantly higher frequency of IgA nephropathy compared to the other genotypes. Although no significant difference in the genotype distribution could be observed between the IgA nephropathy group and thin GBM disease group, this is the first study on the relationship between the development of thin GBM disease and cytokine gene polymorphisms.

### Conflict of interest

No conflict of interest has been declared.

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