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Associations between the *HaeIII* Single Nucleotide Polymorphism in the SLC2A1 Gene and Diabetic Nephropathy in Korean Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic nephropathy (DN) is the most serious microvascular complication of diabetes mellitus and is one of the leading causes of end stage renal failure. In previous studies, the contribution of genetic susceptibility to DN showed inconsistent results. In this study, we investigated the association between the solute carrier family 2 facilitated glucose transporter member 1 (SLC2A1) *HaeIII* polymorphism and DN in Korean patients with type 2 diabetes mellitus (T2DM) according to disease duration.

Methods: A total of 846 patients with T2DM (mean age, 61.3 ± 12.3 years; mean duration of T2DM, 10.3 ± 7.9 years; 55.3% men) who visited the Chungbuk National University Hospital were investigated. The *HaeIII* polymorphism of the SLC2A1 gene was determined by the real time polymerase chain reaction method. Genotyping results were presented as GG, AG, or AA. A subgroup analysis was performed according to duration of T2DM (≤ 10 years, > 10 years).

Results: The AG + AA genotype showed a significantly higher risk of DN compared with the GG genotype in patients with a type 2 DM duration less than 10 years (12.4% vs. 4.2%; $P < 0.001$). No significant differences were observed in terms of other diabetic complications, including retinopathy, peripheral neuropathy, cardiovascular disease, cerebrovascular disease or peripheral artery disease, according to the genotypes of the SLC2A1 *HaeIII* polymorphism.

Conclusion: The SLC2A1 *HaeIII* polymorphism was associated with DN in Korean patients with T2DM, particularly in the group with a relatively short disease duration.

Keywords: Polymorphism; SLC2A1; *HaeIII*; Diabetic Nephropathies; Diabetes Mellitus, Type 2

INTRODUCTION

Diabetes mellitus (DM) has rapidly increased to epidemic proportions over the past few decades due to changes in lifestyle. The prevalence of DM worldwide was estimated to be

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Author Contributions

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8%–10% in 2018. The number of patients with DM is predicted to increase to 550 million cases by 2035.¹

Diabetic nephropathy (DN) is the leading cause of chronic kidney disease and is associated with increased cardiovascular morbidity and mortality.² DN is a serious diabetic microvascular complication that develops in 25%–40% of patients with long-standing DM.³ It has been estimated that more than 20%–40% of patients with DM will be affected by end stage renal disease requiring renal replacement therapy.¹ DN is defined by an increased excretion of urine albumin or a decreased glomerular filtration rate or both. Sustained hyperglycaemia is potentially modifiable and a progression factor for DN.⁴ Clinical trials have consistently demonstrated that intensive glucose control decreases the risk for clinical and structural manifestations of DN in both type 1 and 2 DM.^{5,6} Other reported risk factors for DN include hypertension, men gender, glomerular hyperfiltration, smoking, dyslipidaemia, proteinuria levels and dietary factors.⁷ Although optimal glycaemic and blood pressure control are inversely related to DN, a subset of patients appears to be at increased risk.⁶ In contrast, DN develops in 40% of patients with DM, even when hyperglycaemia is sustained for long periods of time. A racial difference has been detected in the epidemiology of DN.^{8,9} DN is more prevalent among African Americans, Asians and Native Americans than Caucasians.¹⁰ Familial clustering of DN is present in patients with both type 1 and 2 DM.^{11,12} In addition, some genetic loci and polymorphisms in specific genes have been associated with DN.¹³ Taken together, these results raise the possibility that genetic susceptibility may have an impact on the initiation and development of DN. Despite evidence for a genetic component to DN in patients with type 1 and 2 DM, the underlying genetic mechanisms remain poorly understood with no definitive candidate genes yet established.

Glomerulosclerosis is a major pathological finding of DN. Diabetic glomerulosclerosis is characterised by the accumulation of extracellular matrix proteins in the mesangial space with mesangial expansion, and tubulointerstitial fibrosis.¹⁴ Intracellular hyperglycaemia via glucose transporter 1 (GLUT1) induces the expression of cytokines or growth factors associated with the development of glomerulosclerosis.¹⁵

GLUT1, also known as solute carrier family 2 facilitated glucose transporter member 1 (SLC2A1), is a membrane-embedded protein encoded by the SLC2A1 gene.¹⁶ It is responsible for facilitated diffusion of glucose across a membrane. GLUT1 is highly abundant in the mesangial cells of glomeruli. GLUT1-mediated utilisation of glucose in mesangial cells triggers several pro-sclerotic pathways, all of which can induce the development of glomerulosclerosis.¹⁷ Thus, GLUT1 is likely to play a pivotal role in the development of diabetic changes in the kidney. Therefore, SLC2A1 is a candidate gene that might confer susceptibility to DN. Several studies have evaluated the association with the SLC2A1 gene polymorphism, including *Xba*I (rs841853), *Enh*2-1 (rs841847), *Enh*2-2 (rs841848), *Hpy*CH4V (rs710218), and DN.^{18,19} Most studies consisted of a small sample size and have shown discrepant results. In addition, few studies have been conducted on large Asian populations.

To examine whether the SLC2A1 *Hae*III polymorphism is involved in the susceptibility to DN in Korean patients with type 2 DM, we compared the frequency of the SLC2A1 *Hae*III genotype in patients with and without renal complications according to disease duration.

METHODS

Study population

A total of 846 patients with type 2 DM who visited the Chungbuk National University Hospital (CNUH) were included in the analysis. The diagnosis of DM was defined according to World Health Organization criteria. We excluded patients < 30 years, with type 1 DM or other types of DM. Demographic data were collected for each patient, such as age, gender, height, weight, body mass index (BMI), duration of diabetes and family history of DM. Laboratory data, including fasting plasma glucose, hemoglobin A1c (HbA1c), C-peptide, insulin, liver function, kidney function, and lipid metabolism parameters were also collected for each patient.

Diabetic complications

Both microvascular (such as retinopathy, nephropathy, and peripheral neuropathy) and macrovascular (including cardiovascular disease, cerebrovascular disease, and peripheral artery disease) diabetic complications were evaluated. Retinopathy and peripheral neuropathy were assessed based on medical records. Type 2 DM with nephropathy was diagnosed based on overt albuminuria, urinary albumin excretion > 300 mg/24 hr (> 200 µg/min; representing persistent albuminuria) with or without elevated serum creatinine levels (serum creatinine > 1.3 mg/dL), determined on at least two separate occasions 3 months apart, and in the absence of clinical or radiological evidence of non-diabetic renal disease. Cardiovascular disease was evaluated based on medical records and the results of coronary angiographic data. Cerebrovascular disease was also assessed based on medical records and, if possible, magnetic resonance imaging results. Peripheral artery disease was defined as an ankle-brachial index value < 1.20.

Genotyping

The SLC2A1 polymorphism (rs1385129, A/G) was detected using the TaqMan probe-based real-time polymerase chain reaction (PCR). The mixture of PCR primers and fluorescent probes for rs1385129 (Thermo Fisher Scientific, Waltham, MA, USA; TaqMan™ single nucleotide polymorphism (SNP) Genotyping Assay, Cat# 4351379) was used for PCR amplification with a genotyping master mix (Thermo Fisher Scientific, Cat# 4371353) in a 25 µL reaction volume. Reactions were cycled using the following parameters: preheating at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Fluorescence was detected at the end of every cycle, and the genotype data were automatically generated by a BioRad CFX96 Real-Time PCR system (BioRad Laboratories, Hercules, CA, USA).

Statistical analysis

The probability of Hardy–Weinberg equilibrium was tested using the χ^2 test. Data are expressed as mean \pm standard deviation or as percentages for categorical variables. The baseline characteristics were compared using Student's *t*-test for continuous variables and the χ^2 test for categorical parameters. The χ^2 test was used to evaluate differences in prevalence according to genotype. Multiple logistic regression analyses were performed to evaluate the risk factors associated with DN. All statistical analyses were performed using SPSS for Windows software (ver. 22.0; IBM Corp., Armonk, NY, USA). A *P* value < 0.05 was considered significant.

Ethics statement

All participants provided written informed consent. This study was approved by the Institutional Review Board (IRB) of CNUH (IRB No. 2018-03-034-001). The current study was conducted according to the guidelines administered by the Declaration of Helsinki.

RESULTS

Characteristics of the study population

The demographic and biochemical characteristics of the subjects included in this study are described in **Table 1**. The present study included 846 subjects with type 2 DM, comprising 468 men and 378 women. The duration of DM was 10.3 ± 7.9 years, and about half of the patients had a family history of diabetes. The mean age and BMI of the study subjects were 61.3 ± 12.3 years and 25.7 ± 3.8 kg/m², respectively.

The frequencies of the SLC2A1 genotypes in the study subjects were as follows: GG, 62.1% (n = 525); AG, 33.9% (n = 287); and AA, 4.0% (n = 34). The BMIs of the GG and AG + AA groups were 25.5 ± 3.8 kg/m² and 26.2 ± 3.8 kg/m², respectively. The GG group had a lower BMI than that of AG + AA group. The GG group had a longer duration of DM and had a higher family history prevalence than the AG + AA group. The GG group had a higher level of HbA1c than the AG + AA group. The clinical characteristics of the patients with DM duration ≤ 10 years are summarised in **Table 2**. The AG + AA group had a higher prevalence of hypertension than the GG group.

Table 1. Baseline characteristics of the study population

Characteristics	Total (n = 846)	GG (n = 525)	AG + AA (n = 321)	P value ^a
Age, yr	61.3 ± 12.3	61.4 ± 12.4	61.2 ± 12.2	0.881
Gender, men/women	468/378	299/266	169/152	0.222
Height, cm	161.9 ± 9.4	162.2 ± 9.4	161.4 ± 9.5	0.280
Weight, kg	67.7 ± 12.7	67.2 ± 12.6	68.4 ± 12.8	0.193
BMI, kg/m ²	25.7 ± 3.8	25.5 ± 3.8	26.2 ± 3.8	0.012
Duration of DM, yr	10.3 ± 7.9	10.9 ± 7.9	9.3 ± 7.6	0.004
Family history of DM, No (%)	420 (49.6)	275 (52.4)	145 (45.2)	0.042
Hypertension, No. (%)	432 (51.1)	261 (49.7)	171 (53.3)	0.323
HbA1c, %	7.5 ± 1.6	7.6 ± 1.6	7.4 ± 1.5	0.038
FPG, mg/dL	143.0 ± 50.2	144.8 ± 51.8	140.0 ± 47.4	0.183
PP2, mg/dL	217.9 ± 96.5	223.8 ± 107.4	208.2 ± 75.0	0.359
C-peptide, ng/mL	2.0 ± 1.6	2.0 ± 1.3	2.2 ± 1.9	0.067
Insulin, IU/mL	16.2 ± 34.4	15.5 ± 32.2	17.4 ± 37.8	0.464
Glucagon, pg/mL	249.4 ± 163.4	248.7 ± 171.1	250.3 ± 151.8	0.925
HOMA-IR	5.7 ± 13.6	5.6 ± 14.0	5.9 ± 12.6	0.759
HOMA-β	38.0 ± 88.9	34.4 ± 87.3	172.8 ± 123.5	0.208
Total cholesterol, mg/dL	160.7 ± 37.6	161.5 ± 38.5	159.3 ± 36.5	0.400
Triglycerides, mg/dL	154.1 ± 99.5	153.2 ± 104.3	155.6 ± 91.3	0.738
HDL-cholesterol, mg/dL	48.6 ± 13.5	48.9 ± 13.4	48.2 ± 13.6	0.520
LDL-cholesterol, mg/dL	94.3 ± 30.9	95.0 ± 31.6	93.0 ± 29.8	0.372
AST, IU/L	24.9 ± 14.7	24.5 ± 13.3	25.6 ± 16.8	0.264
ALT, IU/L	26.7 ± 19.7	26.3 ± 18.3	27.3 ± 21.8	0.456
BUN, mg/dL	16.3 ± 7.4	16.4 ± 7.7	16.2 ± 7.0	0.684
Creatinine, mg/dL	0.95 ± 0.71	0.95 ± 0.63	0.95 ± 0.83	0.982
Urine ACR, mg/g	198.01 ± 838.67	208.26 ± 778.88	180.82 ± 931.53	0.655

Data are presented as mean ± standard deviation.

BMI = body mass index, DM = diabetes mellitus, HbA1c = hemoglobin A1c, FPG = fasting plasma glucose, PP2 = postprandial 2-hour glucose, HOMA = homeostatic model assessment, IR = insulin resistance, HDL = high-density lipoprotein, LDL = low-density lipoprotein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, ACR = albumin-creatinine ratio.

^aP values were calculated using Student's *t*-test for continuous data and the χ^2 test for categorical data.

Table 2. Clinical characteristics of patients with a DM duration less than 10 years

Characteristics	GG (n = 289)	AG + AA (n = 217)	P value ^a
Age, yr	58.2 ± 13.3	58.8 ± 12.3	0.628
Gender, men/women	161/128	129/88	0.400
Height, cm	162.6 ± 9.9	163 ± 9.0	0.609
Weight, kg	68.2 ± 12.7	69.5 ± 13.6	0.260
BMI, kg/m ²	25.7 ± 3.6	26.0 ± 4.0	0.322
Duration of DM, yr	5.0 ± 2.8	5.0 ± 2.7	0.944
Family history of DM, No. (%)	147 (50.9)	93 (42.9)	0.074
Hypertension, No. (%)	106 (36.9)	107 (49.8)	0.004
HbA1c, %	7.3 ± 1.5	7.2 ± 1.4	0.466
FPG, mg/dL	138.1 ± 46.6	137.5 ± 45.0	0.880
PP2, mg/dL	217.2 ± 106.7	203.5 ± 76.0	0.458
C-peptide, ng/mL	2.0 ± 1.3	2.2 ± 1.8	0.077
Insulin, IU/mL	12.2 ± 21.9	13.2 ± 19.6	0.612
Glucagon, pg/mL	227.8 ± 150.4	229.6 ± 146.5	0.926
HOMA-IR	4.0 ± 6.7	4.6 ± 7.8	0.325
HOMA-β	79.7 ± 645.2	183.6 ± 1,418.9	0.296
Total cholesterol, mg/dL	166.2 ± 40.1	160.3 ± 36.0	0.089
Triglycerides, mg/dL	152.3 ± 103.7	155.7 ± 92.3	0.710
HDL-cholesterol, mg/dL	49.7 ± 13.0	49.1 ± 13.9	0.648
LDL-cholesterol, mg/dL	99.1 ± 34.8	93.2 ± 30.1	0.051
AST, IU/L	26.1 ± 15.3	26.5 ± 19.0	0.820
ALT, IU/L	28.7 ± 20.9	28.5 ± 23.3	0.897
BUN, mg/dL	14.9 ± 6.3	15.6 ± 5.9	0.183
Creatinine, mg/dL	0.83 ± 0.47	0.94 ± 0.83	0.064
Urine ACR, mg/g	60.98 ± 316.68	147.9 ± 992.22	0.233

Data are presented as mean ± standard deviation.

DM = diabetes mellitus, BMI = body mass index, HbA1c = hemoglobin A1c, FPG = fasting plasma glucose, PP2 = postprandial 2-hour glucose, HOMA = homeostatic model assessment, IR = insulin resistance, HDL = high-density lipoprotein, LDL = low-density lipoprotein, AST = aspartate aminotransferase, ALT = alanine aminotransferase, BUN = blood urea nitrogen, ACR = albumin-creatinine ratio.

^aP values were calculated using Student's *t*-test for continuous data and the χ^2 test for categorical data.

SLC2A1 HaeIII polymorphism and DN according to duration of diabetes

The genotypic distribution of SLC2A1 was in accordance with Hardy-Weinberg equilibrium. A subgroup analysis according to the duration of DM showed a significantly higher prevalence of DN in the AG + AA group than the GG group (12.4% vs. 4.2%, $P < 0.001$) in patients with a DM duration ≤ 10 years. The prevalence of DN in patients with DM duration > 10 years tended to increase in both genotype groups (27.1% vs. 20.2%, $P = 0.174$) (Table 3 and Fig. 1).

A multiple logistic regression analysis was performed in patients with a DM duration ≤ 10 years and the results are shown in Table 4. The A allele was significantly associated with a higher risk of DN (odds ratio, 3.339; 95% confidence interval, 1.642–6.788; $P = 0.001$) after adjusting for other clinical parameters.

Table 3. SLC2A1 polymorphisms and diabetic complications according to duration of DM

Variables	Total			Duration (yr)					
				≤ 10			> 10		
	GG (n = 525)	AG + AA (n = 321)	P value	GG (n = 289)	AG + AA (n = 217)	P value	GG (n = 236)	AG + AA (n = 104)	P value
DM nephropathy	76 (14.5)	48 (15.0)	0.849	12 (4.20)	27 (12.4)	0.001	64 (27.1)	21 (20.2)	0.174
DM retinopathy	156 (29.7)	83 (25.9)	0.227	38 (13.1)	33 (15.2)	0.509	118 (50.0)	50 (48.1)	0.744
DM neuropathy	114 (21.7)	69 (21.5)	0.940	44 (15.2)	38 (17.5)	0.490	70 (29.7)	31 (29.8)	0.978
Cardiovascular disease	76 (14.5)	40 (12.5)	0.408	26 (9.0)	24 (11.1)	0.441	50 (21.2)	16 (15.4)	0.213
Cerebrovascular disease	56 (10.7)	25 (7.8)	0.167	27 (9.3)	19 (8.8)	0.820	29 (12.3)	6 (5.8)	0.068
Peripheral artery disease	18 (3.4)	5 (1.6)	0.104	7 (2.4)	2 (0.9)	0.206	11 (4.7)	3 (2.9)	0.443

Data are presented as number (%).

DM = diabetes mellitus.

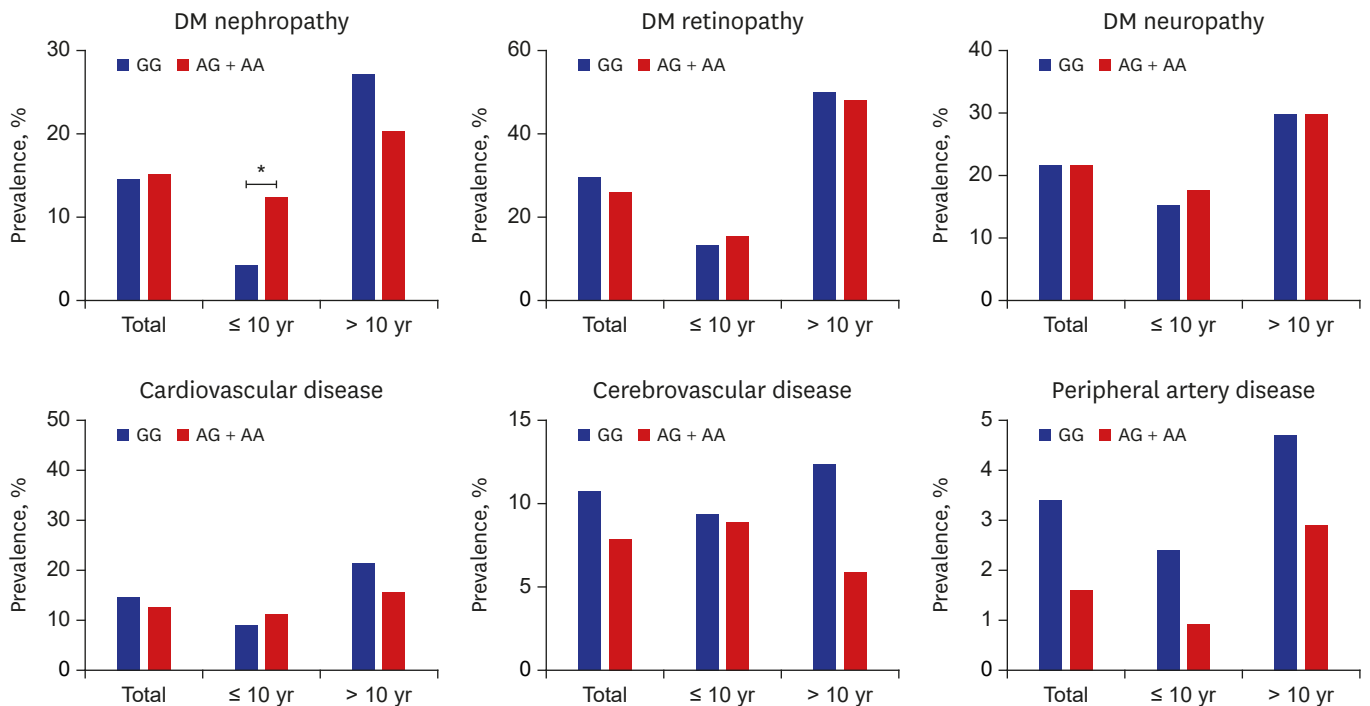


Fig. 1. SLC2A1 genotypes and prevalence of diabetic complications among groups according to the duration of diabetes. DM = diabetes mellitus.

Table 4. Logistic regression analysis of risk factors for the association with DN in patients with DM duration less than 10 years

Risk factors	DN	
	OR (95% CI)	P value
A allele	3.339 (1.642-6.788)	0.001
Age ^a	1.318 (1.001-1.734)	0.049
Gender	1.194 (0.611-2.331)	0.604
HbA1c ^b	1.102 (0.881-1.378)	0.397

DN = diabetic nephropathy, DM = diabetes mellitus, OR = odds ratio, CI = confidence interval, HbA1c = hemoglobin A1c.

^aRisk associated with a 10 years increase in age; ^bRisk associated with a 1% increase in HbA1c.

SLC2A1 HaeIII polymorphism and macrovascular complications

The associations between the SLC2A1 HaeIII polymorphism and diabetic macrovascular complications are presented in Table 3 and Fig. 1. The prevalence rates of macrovascular complications in the whole cohort, including cardiovascular, cerebrovascular disease and peripheral artery disease in the GG and AG + AA groups were 14.5% vs. 12.5%, 10.7% vs. 7.8% and 3.4% vs. 1.6%, respectively. No association was detected between the SLC2A1 HaeIII polymorphism and cardiovascular disease, cerebrovascular disease or peripheral artery disease. A subgroup analysis performed according to disease duration revealed the absence of a significant difference between the two groups regarding macrovascular complications.

SLC2A1 HaeIII polymorphism and other microvascular complications

The prevalence of retinopathy was 29.7% in the GG group and 25.9% in the AG + AA group. The prevalence of neuropathy was also similar between the two groups (21.7% vs. 21.5%). A subgroup analysis that was performed according to disease duration revealed no significant differences between the two groups regarding retinopathy or peripheral neuropathy.

DISCUSSION

The purpose of this study was to elucidate a possible association between genetic variants of the SLC2A1 *HaeIII* polymorphism and DN in 846 Korean patients with type 2 DM according to disease duration. In this study, the AG + AA genotype showed significantly higher risk of DN compared with the GG genotype in patients with a DM duration < 10 years. The incidence of DN in patients with type 2 DM is low during the first 10 years of DM duration, after which it increases rapidly to a maximum at about 18 years of duration.²⁰ Thus, we conducted an analysis on the association between the SLC2A1 *HaeIII* polymorphism and DN according to the duration of DM to exclude the influence of DM duration on DN.

Several studies have considered the role of genetic variants in the SLC2A1 gene in conferring genetic susceptibility to DN. However, the role of the SLC2A1 polymorphism in the development of DN remains controversial. The SLC2A1 *HaeIII* polymorphism is a risk factor for DN in Kurdish and Tunisian patients with type 2 DM.^{21,22} Amini et al.²¹ reported that the high frequency of the C allele of the SLC2A1 *HaeIII* polymorphism in Kurdish patients with type 2 DM, DN, and the CC genotype was strongly correlated with the risk of DN. Along with this finding, they suggested that the higher rate of the C allele may lead to higher uptake of glucose by mesangial cells and this mechanism may accelerate the development of DN. Makni et al.²² showed that the SLC2A1 *HaeIII* polymorphism confers susceptibility to DN in Tunisian patients with type 2 DM. They also identified that the CGT and CAT haplotypes of SLC2A1 play a possible role in the development of DN.

In contrast, Ng et al.²³ reported no association between the SLC2A1 *HaeIII* polymorphism and the development of DN in 556 Caucasian patients with type 1 DM. However, they demonstrated that *XbaI*(-)*XbaI*(-) of SLC2A1 homozygotes was at an increased risk of DN in patients with type 1 DM. They also investigated the association with enhancer-2 SNP 1 (Enh2-1) located in intron 2, at a distance < 2 kb from the *XbaI* polymorphism, and DN in patients with type 1 DM. The results of this study revealed that homozygosity for the A allele of SLC2A1 Enh2-1 was a possible risk factor for the development of DN. They suggested that Enh2-1 SNP 1 could be the causative SLC2A1 polymorphism that directly influences genetic susceptibility to DN because it is located in a putative enhancer region that has potential to modulate gene expression. A total of 846 subjects were evaluated for the SLC2A1 *HaeIII* polymorphism in our study. In comparison, more than 300 subjects were enrolled in their study. It is expected that the SLC2A1 *HaeIII* genotype distribution in our study might be more reliable than previous studies due to the large sample size.

Marques et al.²⁴ detected no association between the SLC2A1 *HaeIII* polymorphism and DN in Brazilian patients with type 1 DM. They identified an association between DN and three SLC2A1 SNPs, such as rs841847 (Enh2-1), rs841848 (Enh2-2) and rs3820589. They found that the AA genotype of rs3820589 SLC2A1 was associated with DN but not rs841847 (Enh2-1) or rs841848 (Enh2-2). However, a haplotype analysis demonstrated that the change in G/A in rs841847 of the AGG/AG haplotype and the change in C/T in rs841847 of the AGGC/T haplotype were associated with DN at baseline, even though they did not find any association between these polymorphisms and DN at baseline. This inconsistent finding might be explained by differences in the populations and the numbers of subjects. The distinct clinical endpoints of DN may have resulted in the discrepant findings. The different pathophysiological features between type 1 and 2 DM could also affect these contrasting findings.

GLUT1 is the major facilitative glucose transporter present in mesangial cells,¹⁷ and is encoded by the SLC2A1 gene. In patients with DM, GLUT1-mediated utilisation of glucose activates signalling cascades, including four pro-sclerotic pathways, all of which can promote the development of glomerulosclerosis in DN.¹⁷ In addition, one study demonstrated a role for GLUT1-induced mTOR in mesangial dysfunction and glomerular disease in patients with DM. GLUT1 activity is considered a rate-limiting factor in the development of diabetic changes in the kidney.²⁵ Genetic changes in GLUT1 expression may promote glomerulosclerosis. Therefore, the SLC2A1 polymorphism may influence GLUT1 expression and the development of DN.

In this study, we also evaluated the association between the SLC2A1 *HaeIII* polymorphism and diabetic retinopathy and diabetic peripheral neuropathy for the first time in Korean patients with type 2 DM. No association was detected between the SLC2A1 *HaeIII* polymorphism and diabetic retinopathy or peripheral neuropathy. Although diabetic microcomplications have common pathophysiological processes, the annual incidence rates of diabetic retinopathy, neuropathy and DN are different. The development of diabetic retinopathy and DN may have organ-specific responses, in addition to common pathophysiological processes. Therefore, the contradictory effect of the SLC2A1 *HaeIII* polymorphism on DN and diabetic retinopathy may be explained by this phenomenon.

GLUT1 is the most important carrier of glucose through the blood-retinal barrier. It is expressed in the vascular endothelial cells of the inner blood-retinal barrier and retinal pigment epithelial cells of the outer blood-retinal barrier.²⁶ Increased GLUT1 expression has been detected in the endothelial cells of patients with diabetic retinopathy²⁷; thus, GLUT1 may have a role in the development of diabetic retinopathy. To the best of our knowledge, no studies have considered the association between the SLC2A1 *HaeIII* polymorphism (rs1385129) and diabetic retinopathy. A study on Malaysian patients with type 2 DM reported no relationship between the SLC2A1 26177A/G polymorphism and diabetic retinopathy.²⁸ SLC2A1 *XbaI* (rs841853) also fails to show any association with diabetic retinopathy in Chinese patients with type 2 DM.²⁹ In contrast, Siokas et al.³⁰ reported that the tag SLC2A1 polymorphism is associated with diabetic retinopathy and DN in Greek patients with type 2 DM. Roy et al.³¹ also showed that the SLC2A1 polymorphism is associated with the progression of diabetic retinopathy in African-Americans with type 1 DM. The reason for the conflicting results is thought to be the differences in sample size, genetic background and population composition.

The present study had some limitations. First, we did not demonstrate GLUT1 expression according to the genotype, as in other polymorphism studies. Second, this study did not demonstrate the influence of the SLC2A1 *HaeIII* polymorphism on the progression of DN or its clinical course. Third, data was collected by reviewing medical records, therefore, there is a possibility of missing data and recall biases. Finally, the study population consisted only of patients with type 2 DM.

However, one of the strengths of this study is that we conducted the analysis according to the duration of DM to exclude the influence of DM duration on DN. Furthermore, it is the first time that the association between the SLC2A1 *HaeIII* polymorphism and DN has been demonstrated in Korean patients with type 2 DM in a relatively large sample size.

In conclusion, our study suggests that the SLC2A1 *HaeIII* polymorphism contributes to the risk of developing DN in Korean patients with type 2 DM.

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