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Manganese superoxide dismutase gene polymorphism (V16A) is associated with stages of albuminuria in Korean type 2 diabetic patients

Seong Jin Lee[a], Moon Gi Choi[a], Dong-Sun Kim[b], Tae Wha Kim[b,*]

[a]*Department of Endocrinology and Metabolism, College of Medicine, Hallym University, ChunCheon 200-704, South Korea*
[b]*Department of Endocrinology and Metabolism, College of Medicine, Hanyang University, Seoul 133-792, South Korea*

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Abstract

Several single-nucleotide polymorphisms of genes related to oxidative stress have been evaluated because intracellular reactive oxygen species are associated with development of diabetes and its microvascular complications. We performed a case-control study to investigate whether V16A polymorphism of manganese superoxide dismutase (Mn-SOD) gene is related to pathogenesis of diabetes and whether the polymorphism is associated with stages of albuminuria in Korean type 2 diabetic patients. Genotype distributions were studied in 178 nondiabetic subjects and 371 type 2 diabetic patients of 3 groups with a normoalbuminuria group (Normo group, n = 244), a microalbuminuria group (Micro group, n = 86), and an overt albuminuria group (Macro group, n = 41). The albumin/creatinine ratio (ACR) was defined as a urinary albumin/creatinine ratio. V16A genotypes were determined with polymerase chain reaction–restriction fragment length polymorphism method. Between nondiabetic subjects and type 2 diabetic patients, Mn-SOD genotype distribution (VV/VA + AA, 146/32 vs 314/57) and A allele frequency (0.121 vs 0.104) were not different. Patients with nephropathy, Micro and Macro groups, had significantly lower A allele frequency, longer diabetic duration, higher prevalence of hypertension, and greater ACR than those of patients without nephropathy ($P < .05$). A allele was significantly less frequent with progression of nephropathy (Normo group, 0.119; Micro group, 0.073; Macro group, 0.03; $P < .05$). In type 2 diabetic patients, A allele carriers had significantly lower prevalence of hypertension and lesser ACR than those of A allele noncarriers ($P < .01$). In multivariate analysis, hypertension, duration of diabetes, serum total cholesterol level, and A allele of Mn-SOD gene were independently associated with stages of albuminuria. These results suggest that V16A polymorphism of Mn-SOD gene is not related to pathogenesis of diabetes but is associated with stages of albuminuria in Korean type 2 diabetes.

1. Introduction

The development of diabetic nephropathy is influenced by genetic factors as well as glycemic control. Therefore, early detection of patients who have genetically increased susceptibility to diabetic nephropathy may provide a great benefit in treatment for diabetic nephropathy. In regard to genetic analysis, several single-nucleotide polymorphisms of genes related to oxidative stress, an imbalance between intracellular reactive oxygen species (ROS) and antioxidants, have been investigated because ROS including superoxide radical, hydrogen peroxide, and hydroxyl radical are associated with initiation and progression of diabetic nephropathy, but very few polymorphic variants have been reported to be clinically significant [1].

Manganese superoxide dismutase (Mn-SOD) serves as an essential defender against mitochondrial superoxide radical [2]. Mn-SOD translates in cytosol and translocates into mitochondria by signal peptide, which plays a key role in targeting the enzyme to mitochondria. *Sod2* gene encoding Mn-SOD is located in chromosome 6q25 [2], and valine/alanine polymorphic site described as "Val(16)Ala" or "V16A" has been recently identified at the 16th amino acid position in the second exon [3]. It is also known as "Val(−9)Ala" because of the ninth amino acid sequence in signal peptide [4]. Previous study suggested

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* Corresponding author. Department of Endocrinology and Metabolism, College of Medicine, Hanyang University, Seoul 133-792, South Korea. Tel.: +82 2 2290 8325; fax: +82 2 2290 8305.

E-mail address: kimth@hanyang.ac.kr (T.W. Kim).

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that ROS scavenging capacity may be closely related to translocated amounts of Mn-SOD by V16A polymorphism of Mn-SOD gene [5].

Considering that V16A polymorphism affects Mn-SOD activity which influences the severity of diabetic nephropathy related to oxidative stress, it is theoretically possible that V16A polymorphism may be associated with severity of diabetic nephropathy. Recently, 2 studies reported on the relationship between V16A polymorphism and diabetic nephropathy in Russian type 1 and in Japanese type 2 diabetic patients, but the relationship still remains unclear because of their contradictory results [5,6].

The present study was designed to investigate whether V16A polymorphism of Mn-SOD gene is related to pathogenesis of diabetes and whether the polymorphism is associated with stages of albuminuria in Korean type 2 diabetic patients. We simultaneously analyzed the insertion (I)/deletion (D) polymorphism of angiotensin-converting enzyme (ACE) gene in the 16th intron for avoidance of its confounding effect.

2. Subjects and methods

All subjects were Korean. Type 2 diabetic patients were enrolled among outpatients of Hallym Medical Center, ChunCheon Sacred Heart Hospital, from September 2002 to December 2003. Nondiabetic subjects were selected from visitors in the health care center of the hospital. Informed written consent was obtained from all subjects before enrollment. Study protocol was designed according to the Declaration of Helsinki and was approved by the Ethics Committee of ChunCheon Sacred Heart Hospital. Type 2 diabetes was diagnosed as a serum glucose level at fasting or postprandial 2 hours of more than 126 or 200 mg/dL, respectively, and a serum C-peptide level at fasting of more than 1.0 ng/mL on 2 separate occasions. Fasting plasma glucose (coefficient of variation [CV], 1.1%), glycosylated hemoglobin (HbA1c) (CV, 1.2%), serum total cholesterol (CV, 0.5%), triglyceride (CV, 0.7%), and creatinine (CV, 1.1%) levels were determined by routine automated laboratory methods. Hypertension was defined as a systolic blood pressure (SBP) greater than or equal to 140 mm Hg or a diastolic blood pressure (DBP) greater than or equal to 90 mm Hg or under antihypertensive treatment. Cases of age more than 70 years, nondiabetic renal problems, chronic liver disease, pregnancy, heavy alcoholic, glucocorticoid medication, and urinary tract infection were excluded.

Albuminuria was diagnosed as a urinary albumin/creatinine ratio (ACR) which was determined based on the average of at least 2 separate measurements. After pretest urination, patients were asked to avoid exercise for 1 hour, and spot urine was collected followed by measurement of urinary albumin concentration by radioimmunoassay. Urinary creatinine concentration was also measured with modified Jaffe method. By ACR, type 2 diabetic patients were divided into 3 groups: a normoalbuminuria group (Normo group, urinary ACR <30 mg/g Cr), a microalbuminuria group (Micro group, urinary ACR between 30 and 300 mg/g Cr), and an overt albuminuria group (Macro group, urinary ACR >300 mg/g Cr).

Type 2 diabetic patients were treated with diet alone (126 kJ/kg standard body weight per day containing 60% carbohydrate and 25% fat), or with diet in combination with oral hypoglycemic agents (sulfonylurea, biguanide, α-glucosidase inhibitor, thiazolidinedione), or with diet in combination with insulin therapy.

2.1. Genotyping

Genomic DNA was extracted from peripheral polymorphonuclear cells using a DNA extraction kit (Bioneer DNA Extraction Kit, Bioneer, Seoul, South Korea). In brief, 20 μL proteinase K was added to 200 μL of whole blood, and the sample was incubated at 60°C for 10 minutes with 200 μL of binding buffer. After 100 μL of isopropanol was mixed, the sample was transferred onto binding column tube and was centrifuged at 8000 rpm for 1 minute. Washing and elution were carried out, and genomic DNA was preserved at −70°C.

V16A genotypes of Mn-SOD gene were determined with polymerase chain reaction (PCR)–restriction fragment length polymorphism method. DNA fragment containing V16A polymorphic site was amplified from genomic DNA by PCR with a forward primer (F1: 5′-GCTGTGCTTCTCGTCTTC-AG-3′) and a reverse primer (R1: 5′-TGGTACTTCTCCTC-GGTGACG-3′) at 38 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. V16A genotyping by RFLP was performed. The PCR product was digested for 2 hours at 60°C with *Bsa*wI restriction enzyme (New England Biolabs, Beverly, Mass). C→T substitution at the 16th nucleotide creates *Bsa*wI digestion site, and digestion of the PCR product (207-bp fragment) by *Bsa*wI makes 163- and 44-bp fragments (Fig. 1). Fragments were separated by electrophoresis on 1.0% agarose gel and were identified by staining with ethidium bromide.

After polymorphic region of ACE gene was amplified by PCR with a forward primer (F2: 5′-CTGGAGACCACTCC-CATCCTTTCT-3′) and a reverse primer (R2: 5′-GATG-TGGCCATCACATTCGTCAGAT-3′), the PCR product was electrophoresed on 1.0% agarose gel and was stained with ethidium bromide. I and D alleles were detected as a band of 490 and 190 bp, respectively. All genotypes were confirmed by 2 investigators who did not know the origin of the genomic DNA.

2.2. Statistical analysis

Data are shown as mean ± SD. Statistical analysis was performed using SPSS version 10.0 program (SPSS, Chicago, Ill). Comparisons of clinical and laboratory characteristics between nondiabetic subjects and type 2 diabetic patients were done by unpaired Student *t* test or χ^2 test as appropriate, and skewed data were logarithmically transformed before analysis. Genotype distributions and

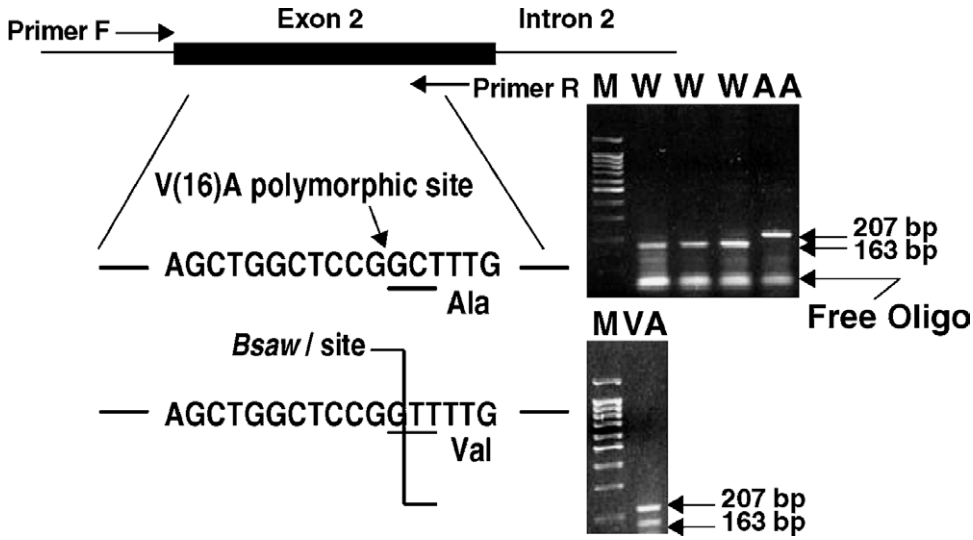

Fig. 1. V16A genotyping of Mn-SOD gene by polymerase chain reaction–restriction fragment length polymorphism method. M indicates marker.

allele frequencies of Mn-SOD and ACE genes in all subjects were analyzed by χ^2 test. Basic characteristics, genotype distributions, and allele frequencies of Mn-SOD and ACE genes among groups according to stages of albuminuria were compared by 1-way analysis of variance test. Tests for Hardy-Weinberg equilibrium were performed by using χ^2 test. A P value less than 5% was considered to be significant. For determination of independent risk factors associated with stages of albuminuria, multivariate analysis was conducted with multiple logistic regression, in which only variables showing a significant association with stages of albuminuria in univariate analysis ($P < .05$) were introduced. Odds ratio (OR) and 95% confidence interval (95% CI) were also estimated.

Table 1
Basic characteristics of nondiabetic subjects (control subjects) and type 2 diabetic patients (diabetic patients)

	Control subjects	Diabetic patients	P
Number (n)	178	371	
Age (y)	51.3 ± 12.7	53.1 ± 13.4	NS
Sex (M/F)	102/76	223/148	NS
BMI (kg/m^2)	23.3 ± 2.8	23.7 ± 3.1	NS
Hypertension, n (%)	56 (31.5)	127 (34.2)	NS
SBP (mm Hg)	127 ± 14	131 ± 18	NS
DBP (mm Hg)	82 ± 11	84 ± 13	NS
FPG (mg/dL)	97.4 ± 8.9	143.1 ± 12.6	<.01
HbA1c (%)	5.7 ± 0.9	8.5 ± 0.9	<.01
Total cholesterol (mg/dL)	207.0 ± 6.1	209.0 ± 5.8	NS
Triglyceride (mg/dL)	203.0 ± 13.2	207.0 ± 14.1	NS
HDL cholesterol (mg/dL)	54.1 ± 5.3	52.2 ± 4.9	NS
Serum creatinine (mg/dL)	0.7 ± 0.3	0.7 ± 0.4	NS
Mn-SOD genotype			
VV, n (%)	146 (82.0)	314 (84.6)	
VA + AA, n (%)	32 (18.0)	57 (15.4)	
A allele frequency	0.121	0.104	NS
ACE genotype			
II, n (%)	74 (41.6)	162 (43.7)	
ID + DD, n (%)	104 (58.4)	209 (56.3)	
D allele frequency	0.343	0.342	NS

Data are mean ± SD.
HDL indicates high-density lipoprotein; NS, not significant.

3. Results

Among the initially enrolled cases of 187 nondiabetic subjects and 382 type 2 diabetic patients, 178 nondiabetic

Table 2
Comparison of characteristics between the groups with and without albuminuria

	Albuminuria (−)	Albuminuria (+)
Number (n)	244	127
Age (y)	53.9 ± 12.1	53.2 ± 13.4
Sex (M/F)	137/117	86/41
Duration of DM (y)	7.3 ± 2.1	10.5 ± 3.8*
BMI (kg/m^2)	23.6 ± 2.8	23.7 ± 3.1
Hypertension, n (%)	67 (27.5)	60 (35.7)*
SBP (mm Hg)	130 ± 14	132 ± 18
DBP (mm Hg)	84 ± 11	85 ± 14
FPG (mg/dL)	142 ± 37	167 ± 65
HbA1c (%)	8.2 ± 0.7	8.6 ± 1.1
Total cholesterol (mg/dL)	201.0 ± 5.8	211.0 ± 5.3
Triglyceride (mg/dL)	191.0 ± 14.8	208.3 ± 19.4
HDL cholesterol (mg/dL)	53.6 ± 3.9	50.9 ± 5.1
Serum creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.3
ACR (mg/g Cr)	12.2 ± 10.6	574.7 ± 408.9*
Retinopathy (NDR/NPDR/PDR)	158/74/12	51/43/33*
Therapy (D/O/I)	45/171/28	16/88/25
Mn-SOD genotype		
VV, n (%)	203 (83.2)	111 (87.4)
VA + AA, n (%)	41 (16.8)	16 (12.6)
A allele frequency	0.119	0.075*
ACE genotype		
II, n (%)	103 (42.2)	59 (46.5)
ID + DD, n (%)	141 (57.8)	68 (53.5)
D allele frequency	0.353	0.323

Data are mean ± SD.
NDR indicates nondiabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; D, diet alone; O, oral hypoglycemic agent; I, insulin.
* $P < .05$ vs albuminuria (−) group.

Table 3
Clinical and laboratory characteristics of type 2 diabetic patients classified by stages of albuminuria

	Albuminuria (−) Normo group	Alb (+)	
		Micro group	Macro group
Number (n)	244	86	41
Age (y)	53.9 ± 12.1	52.1 ± 12.9	54.8 ± 12.3
Sex (M/F)	137/117	59/27	27/14
Duration of DM (y)	7.3 ± 2.1	8.9 ± 2.6*	11.4 ± 3.7*·**
BMI (kg/m^2)	23.6 ± 2.8	23.7 ± 2.9	23.8 ± 2.9
Hypertension, n (%)	67 (27.5)	34 (39.5)*	26 (63.4)*·**
SBP (mm Hg)	130 ± 14	131 ±15	133 ± 15
DBP (mm Hg)	84 ± 11	85 ± 11	85 ± 12
FPG (mg/dL)	142 ± 37	157 ± 41	172 ± 58
HbA1c (%)	8.2 ± 0.7	8.5 ± 0.9	8.7 ± 0.9
Total cholesterol (mg/dL)	201.0 ± 5.8	204.0 ± 4.6	216.0 ± 4.7*·**
Triglyceride (mg/dL)	191.0 ± 14.8	203.0 ± 17.1	214.0 ± 15.2*·**
HDL cholesterol (mg/dL)	53.6 ± 3.9	51.1 ± 4.8	5.4 ± 3.4
Serum creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.2
ACR (mg/g Cr)	12.2 ± 10.6	141.9 ± 98.3*	647.5 ± 382.3*·**
Retinopathy (NDR/NPDR/PDR)	158/74/12	45/22/19***	6/21/14***·****
Therapy (D/O/I)	45/171/28	13/63/12*	3/25/13*·**
Mn-SOD genotype			
VV, n (%)	203 (83.2)	75 (87.2)	36 (87.8)
VA + AA, n (%)	41 (16.8)	11 (12.8)	5 (12.2)
A allele frequency	0.119	0.073*	0.030*·**
ACE genotype			
II, n (%)	103 (42.2)	42 (48.8)	17 (41.5)
ID + DD, n (%)	141 (57.8)	44 (51.2)	24 (58.5)
D allele frequency	0.353	0.320	0.329

Data are mean ± SD.
* $P < .05$ vs Normo group.
** $P < .05$ vs Micro group.
*** $P < .01$ vs Normo group.
**** $P < .01$ vs Micro group.

subjects (102 men and 76 women, 51.3 ± 2.7 years) and 371 type 2 diabetic patients (223 men and 148 women, 53.1 ± 3.4 years) were finally included in this study.

Basic characteristics of nondiabetic subjects and type 2 diabetic patients are summarized in Table 1. There was no difference in age, sex, body mass index (BMI), prevalence of hypertension, SBP and DBP, serum lipid profile, and serum creatinine level between the 2 groups, but fasting plasma glucose (FPG) and HbA1c levels in type 2 diabetic patients were significantly higher than those in nondiabetic subjects ($P < .01$). Mn-SOD genotype distribution and allele frequency were as follows: nondiabetic subjects vs type 2 diabetic patients: genotype distribution (VV/VA + AA), 146 (82.0%)/32 (18.0%) vs 314 (84.6%)/57 (15.4%); allele frequency (V/A), 0.879/0.121 vs 0.896/0.104. Mn-SOD genotype distribution was consistent with Hardy-Weinberg equilibrium ($P = .78$). Overall Mn-SOD genotype distribution and A allele frequency did not differ between the 2 groups. Similarly, ACE genotype distribution and D allele frequency in type 2 diabetic patients were not different from those in nondiabetic subjects. When nondiabetic subjects were divided into normotensive (n = 122) and hypertensive (n = 56) groups, Mn-SOD and ACE genotype distributions (VV/VA + AA, 99 [81.1%]/23 [18.9%] vs 47 [83.9%]/9 [16.1%]; II/ID + DD, 50 [41.0%]/72 [59.0%] vs 24 [42.9%]/32 [57.1%]) as well as A and D allele frequencies (V/A, 0.872/0.128 vs 0.892/0.108; I/D, 0.651/0.349 vs 0.670/0.330) in normotensive group did not differ from those in the hypertensive group.

Patients with albuminuria (albuminuria [+] group, n = 127), Micro and Macro groups, had significantly longer diabetic duration, higher prevalence of hypertension, greater ACR, and more retinopathy than those of patients without albuminuria (albuminuria (−) group, n = 244) ($P < .05$) (Table 2). In comparison of Mn-SOD genotype distribution and A allele frequency between albuminuria (−) and (+) groups, A allele frequency in the albuminuria (−) group was significantly different from that in the albuminuria (+) group (0.119 vs 0.075; $P < .05$). D allele frequency did not differ between the albuminuria (−) and (+) groups ($P = .34$). As shown in Table 3, Mn-SOD genotype distribution of type 2 diabetic patients was in Hardy-Weinberg equilibrium ($P = .61$). The Macro group had significantly longer diabetic duration, higher prevalence of hypertension, and greater ACR than those of the Micro group ($P < .05$). Serum total cholesterol and triglyceride levels were significantly higher in the Macro group than in the Normo or Micro group ($P < .05$). In addition, retinopathy (Normo group, 35.2%; Micro group, 47.7%; Macro group, 85.4%) and proliferative retinopathy (Normo group, 4.9%; Micro group, 22.1%; Macro group, 34.2%) were all significantly more frequent according to stages of

Table 4
Characteristics of type 2 diabetic patients according to carriers or noncarriers of A allele of Mn-SOD gene

	V/V	V/A and A/A	*P*
Number (n)	314	57	NS
Age (y)	52.8 ± 12.3	53.3 ± 12.7	NS
Sex (M/F)	193/121	36/21	NS
Duration of DM (y)	8.7 ± 2.6	9.3 ± 2.8	NS
BMI (kg/m^2)	23.8 ± 3.3	23.6 ± 3.4	NS
Hypertension, n (%)	122 (38.9)	17 (29.8)	<.01
SBP (mm Hg)	131 ±19	130 ± 14	NS
DBP (mm Hg)	85 ± 16	84 ± 13	NS
FPG (mg/dL)	157 ± 51	153 ± 44	NS
HbA1c (%)	8.4 ± 1.1	8.3 ± 0.9	NS
Total cholesterol (mg/dL)	209.0 ± 6.6	203.0 ± 5.4	NS
Triglyceride (mg/dL)	203.0 ± 19.5	201.0 ± 17.1	NS
HDL cholesterol (mg/dL)	53.3 ± 4.6	51.6 ± 4.2	NS
Serum creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	NS
ACR (mg/g Cr)	52.2 ± 31.7	36.9 ± 24.6	<.01

Data are mean ± SD.

Table 5
Multivariate analysis of risk factors associated with stages of albuminuria in type 2 diabetic patients

Variables	OR	95% CI	P
Hypertension	3.17	1.87-4.99	.007
Duration of diabetes	1.04	1.02-1.07	.021
Total cholesterol	1.03	1.01-1.08	.022
A allele	0.47	0.32-0.98	.028

albuminuria ($P < .01$). Therapeutic regimen consisted of diet alone (Normo group, 18.4%; Micro group, 15.1%; Macro group, 7.3%; $P < .05$), or diet with oral hypoglycemic agents (Normo group, 70.1%; Micro group, 73.3%; Macro group, 61.0%; $P = .31$), or diet with insulin (Normo group, 11.5%; Micro group, 11.6%; Macro group, 31.7%; $P < .05$). Although there was no difference in D allele frequency among the groups, A allele was significantly less frequent with stages of albuminuria (Normo group, 0.119; Micro group, 0.073; Macro group, 0.03; $P < .05$). In type 2 diabetic patients, A allele carriers had significantly lower prevalence of hypertension and lesser ACR than those of A allele noncarriers ($P < .01$) (Table 4). Between A allele carriers (n = 57) and A allele noncarriers (n = 314), Normo, Micro, and Macro groups were as follows: A allele carriers vs A allele noncarriers: 41 (71.9%)/11 (19.3%)/5 (8.8%) vs 203 (64.6%)/75 (23.9%)/36 (11.5%).

Multivariate analysis showed that hypertension (OR, 3.17; 95% CI, 1.87-4.99; $P = .007$), duration of diabetes (OR, 1.04; 95% CI, 1.02-1.07; $P = .021$), serum total cholesterol level (OR, 1.03; 95% CI, 1.01-1.08; $P = .022$), and A allele of Mn-SOD gene (OR, 0.47; 95% CI, 0.32-0.98; $P = .028$) were independently associated with stages of albuminuria (Table 5).

4. Discussion

Under normal physiological conditions, mitochondrial ROS play an important role in the defense against bacteria [7-9], but excessive amounts are immediately scavenged by Mn-SOD, which is an essential enzyme for cell survival [10,11]. Diabetes induces overproduction of mitochondrial ROS by activation of electron transport system [12], and imbalance between endogenous prooxidative and antioxidative systems by hyperglycemia contributes to progression as well as initiation of diabetic microvascular complications [13]. Mitochondrial ROS are abruptly increased in both liver and kidney, but Mn-SOD is increased only in liver with no change or decrease in the rest tissues of streptozotocin-induced diabetic rat [14]. It suggests that mitochondrial oxidative reaction may cause oxidative damage in kidney at an early stage of diabetes.

Human Mn-SOD has a tetrameric interface of 2 four-helix bundles [15]. Each subunit is composed of N-terminal helical hairpin and C-terminal α/β domain. Amino acid sequences of Mn-SOD among species are highly preserved, and homologies between human and rodents, and between rat and mouse are 93% and 96%, respectively [3]. This conservation of specific amino acid sequences in Mn-SOD may be essential to maintain enzymatic function.

Sod2 gene encoding Mn-SOD is composed of 5 exons interrupted by 4 introns with typical splice junctions [16]. Two sequence discrepancies resulting in polymorphism of the *Sod2* gene, V16A and I58T, are identified at the 16th and 58th amino acid positions [3]. Structurally altered Mn-SOD variant with reduced activity produces not only early selective benefits through enhanced cytotoxic effects of tumor necrosis factor-α on virus-infected cells but also detrimental influences by increased mitochondrial oxidative stress contributing to diabetes [15]. In I58T polymorphism, Mn-SOD with threonine has a weaker antioxidant activity than that with isoleucine [3]. Pociot et al [17] suggested that I58T polymorphic locus may be a candidate site for diabetic susceptibility in insulin-dependent diabetes. In regard to V16A polymorphism, valine-to-alanine substitution changes a 3-dimensional structure of signal peptide, and thereby the enzymatic content of intramitochondrial compartment is increased based on the condition of the signal peptide with alanine [18]. Decreased A allele frequency is reported in various conditions such as schizophrenia, cardiomyopathy, and asbestosis [18-20]. It means that A allele may be an active type for scavenging of ROS in mitochondria, and thus it may prevent ROS-related pathological processes. Because oxidative stress is likely to be associated with diabetes, it is important to evaluate whether V16A polymorphism related to translocated amounts of Mn-SOD influences the pathogenesis of diabetes and its microvascular complications.

In previous studies, Chistyakov et al [6] reported that A allele frequency was not different between Russian type 1 diabetic patients and nondiabetic subjects, and Nomiyama et al [5] demonstrated similar results in Japanese type 2 diabetic patients, although V allele frequency was higher in Japanese than in Russian nondiabetic subjects (85.6% vs 34.1%). Considering that allele frequency is often influenced by characteristics of study population, discrepancy in allele frequency between the 2 previous studies may be due to racial or geographic difference. In the present study, we investigated whether V16A polymorphism is related to development of diabetes, and the polymorphism is associated with stages of albuminuria in Korean type 2 diabetic patients. Frequency of A allele did not differ between nondiabetic subjects and type 2 diabetic patients, and it suggests that V16A polymorphism is not related to development of type 2 diabetes, at least, in Korean population. Furthermore, these results strongly indicate that V16A polymorphism may not be consistently involved in pathogenesis of diabetes according to different ethnic groups and diabetic types. We also simultaneously analyzed the I/D polymorphism of ACE gene in the 16th intron for

avoidance of its confounding effect. Frequency of D allele was not different between the 2 groups, and thus the I/D polymorphism of ACE gene did not influence our results.

In this study, the albuminuria (+) group had lower A allele frequency as well as longer diabetic duration, higher prevalence of hypertension, and greater ACR than those of the albuminuria (−) group. Diabetic duration, prevalence of hypertension, serum total cholesterol and triglyceride levels, ACR, and A allele frequency of the Macro group were also different from those of the Micro group. A allele carriers had lower prevalence of hypertension and lesser ACR than those of A allele noncarriers. These data show that V16A polymorphism is a possible genetic factor for determining stages of albuminuria. Because retinopathy including proliferative stage was more frequent according to stages of albuminuria in the current study, relationship between retinopathy and genetic factors such as V16A polymorphism should be further investigated.

Multivariate analysis demonstrated that duration of diabetes, hypertension, serum total cholesterol level, and A allele of Mn-SOD gene were independently associated with stages of albuminuria. In addition, genotype distributions and allele frequencies between normotensive and hypertensive nondiabetic subjects were not different. These results also indicate that A allele itself may be associated with stages of albuminuria independently of hypertension. Because association between stages of albuminuria and hypertension (OR, 3.17) is stronger than that of diabetic duration (OR, 1.04), we may be able to prevent progression of albuminuria in normoalbuminuric patients by blood pressure control. We examined I/D polymorphism of ACE gene and found that proportion of D allele carriers was similar among the groups, although the polymorphism was related to progression of albuminuria in other studies [21-23]. These results reveal that association of V16A polymorphism of Mn-SOD gene with stages of albuminuria may be independent of I/D polymorphism of ACE gene in Korean type 2 diabetes.

Prevalence of hypertension was not different between nondiabetic and type 2 diabetic subjects in the current study, although it is usually higher in diabetic patients. It seems to be related to short-term duration of diabetes compared with other studies. Because the present study is cross-sectional and patients with normoalbuminuria had a shorter duration of diabetes, it is possible that normoalbuminuric patients will not always maintain the same stage. Another limitation is that Mn-SOD activity was not measured. Moreover, no evidence has been reported that V16A polymorphism alters oxidative stress in the diabetic patients. It should therefore be evaluated to confirm the biological difference in accordance with V16A polymorphic variant, and further large-scale, cross-sectional, and prospective studies are required to clarify the pathophysiological association of V16A polymorphism with albuminuria. It may be valuable also to investigate the association of I58T polymorphism with development of nephropathy.

In conclusion, our results suggest that V16A polymorphism of Mn-SOD gene is not related to pathogenesis of diabetes but is associated with stages of albuminuria in Korean type 2 diabetic patients.

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