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Association of manganese superoxide dismutase gene polymorphism (V16A) with diabetic macular edema in Korean type 2 diabetic patients

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Abstract

This study was designed to investigate whether V16A polymorphism of the manganese superoxide dismutase (Mn-SOD) gene is associated with the development of type 2 diabetes mellitus and with progression of diabetic retinopathy (DR) and diabetic macular edema (DME). We simultaneously analyzed insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene in the 16th intron to avoid its confounding effect. A total of 192 nondiabetic subjects and 304 type 2 diabetic patients were included in the study. Diabetic retinopathy was classified as nonretinopathy, nonproliferative retinopathy, and proliferative retinopathy. Diabetic macular edema was defined as thickening of the retina and/or hard exudates within a 1-disk diameter of the center of the macula. Diabetic macular edema was further classified into focal, diffuse, and ischemic types. The A allele frequency of the Mn-SOD gene was not different between nondiabetic and type 2 diabetic subjects, between the normotensive and hypertensive groups, between the DR (−) and DR (+) groups, and among the stages of DR. In the DR (+) group, the DME (+) group had a lower A allele frequency than that of the DME (−) group. In the DME (+) group, focal, diffuse, and ischemic types were found in 8, 23, and 6 patients, respectively. The A allele frequency of each type was 0.188, 0.109, and 0.0. The D allele frequency of the angiotensin-converting enzyme gene did not differ in any of the comparisons. Clinical and laboratory parameters of the A allele carriers were not different from those of the noncarriers except for the prevalence of hypertension and DME. Hypertension, diabetic duration, and insulin therapy were related to DR. The A allele, hypertension, and insulin therapy were associated with DME. In conclusion, our results suggest that V16A polymorphism of the Mn-SOD gene is not related to the development of diabetes and progression of DR, but is associated with DME in Korean type 2 diabetic patients.

1. Introduction

Mitochondrial reactive oxygen species play an important role in defense against bacteria under normal physiologic conditions. However, antioxidative enzymes immediately scavenge excessive amounts of mitochondrial reactive oxygen species. Diabetes induces overproduction of mitochondrial reactive oxygen species by hyperactivation of the electron transport system [1], and imbalance between endogenous prooxidative and antioxidative systems by chronic hyperglycemia contributes to progression as well as initiation of diabetic microvascular complications [2].

In diabetes, visual loss primarily results from 2 ocular complications: diabetic retinopathy (DR) and diabetic macular edema (DME). Diabetic retinopathy, a serious microvascular complication of diabetes, is the most common cause of new-onset blindness in Korea. Kowluru et al [3] demonstrated that enzymatic mechanisms involved in protection against oxidative stress were impaired in the retina of diabetic rat, and that oxidative stress played a major role in development and persistence of DR even after adequate glycemic control [4]. Loss of pericyte is another phenomenon of DR, and increased oxidative stress with reduced antioxidative defense may be critical for apoptosis

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of pericyte that occurs in diabetes [5]. Diabetic macular edema is a pathologic response to disruption of the normal permeability barrier named the *blood-retinal barrier* that protects and maintains retinal homeostasis. Diabetes results in DME, which often precedes development of DR [6]. Diabetic macular edema can occur at any time during the progression of DR [7,8]. The severity of DME is not always correlated with the severity of DR. In this regard, Thompson and Ip [9] described that some risk factors were more directly linked to DME, whereas others were more directly linked to DR.

Development of diabetic ocular complications can be influenced by several factors such as genetic variation, glycemic control, concomitant hypertension, and diabetic duration. Several studies reported that genetic factors might play a role in pathogenesis of DR and DME [10-12]. Early detection of patients genetically susceptible to DR and DME may provide substantial treatment benefits. In regard to genetic analysis, single nucleotide polymorphisms of the genes related to oxidative stress have been investigated because reactive oxygen species are associated with the initiation and progression of DR and DME, but very few polymorphic variants have been shown to be clinically significant.

Among antioxidative enzymes, manganese superoxide dismutase (Mn-SOD) serves as an essential defender against mitochondrial superoxide radicals [13]. Mn-SOD translates in cytosol and translocates into the mitochondria by a signal peptide, which plays a key role in targeting the enzyme to mitochondria. The *Sod2* gene encoding Mn-SOD is located in chromosome 6q25.2, and the valine/alanine polymorphic site described as "Val(16)Ala" or "V16A" has been identified at the 16th amino acid position in the second exon [14]. It is also known as "Val(-9)Ala" because of the ninth amino acid sequence in the signal peptide [15]. The A allele frequency decreases in various pathologic conditions such as schizophrenia, cardiomyopathy, and asbestosis [16-18]. This suggests that the A allele is an active type for scavenging of reactive oxygen species in the mitochondria and prevents reactive oxygen species–related pathologic processes. In regard to diabetic microvascular complications, 2 studies revealed the relationship between V16A polymorphism and diabetic nephropathy in Russian type 1 diabetic patients and in Japanese type 2 diabetic patients [19,20]. Recently, we reported that V16A polymorphism was associated with stages of albuminuria in Korean type 2 diabetic patients [21]. Chistyakov et al [20] demonstrated no relationship between V16A polymorphism and DR in Russian type 1 diabetic patients, but the relationship remains unclear. Moreover, the association of V16A polymorphism with DME has not yet been studied.

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

2. Subjects and methods

2.1. Study population

A cross-sectional, case-control study was conducted. All subjects were Korean. Type 2 diabetic patients were enrolled among outpatients of the Hallym Medical Center, ChunCheon Sacred Heart Hospital (ChunCheon, South Korea) from September 2002 to March 2004. Nondiabetic subjects were selected from visitors to the health care center of the same hospital. The study protocol was designed according to the Declaration of Helsinki and was approved by the ethics committee of the ChunCheon Sacred Heart Hospital. Informed written consent was obtained from all subjects before enrollment. Patients who had such changes of plasma glucose and serum lipid levels as Δglucose of 10 mg/dL or less, Δglycosylated hemoglobin (HbA_{1c}) of 1.0% or less, and Δtotal cholesterol of 20 mg/dL or less during the year before enrollment were recruited. Cases of those older than 70 years, with nondiabetic retinal or renal problem, chronic liver disease, pregnancy, heavy alcohol use, glucocorticoid medication, and acute infection were excluded. The subjects of this study partially overlapped with those of the previous study [21].

Type 2 diabetes mellitus was diagnosed as a plasma glucose level at fasting or 2 hours postprandial of greater than 126 or 200 mg/dL and a serum C-peptide level at fasting exceeding 1.0 ng/mL on 2 separate occasions. Hypertension was defined as a systolic blood pressure of $\geq$140 mm Hg or a diastolic blood pressure of $\geq$90 mm Hg or under antihypertensive treatment. Duration of diabetes was considered as the time from which the patient was diagnosed with diabetes. Among the initially enrolled cases of 208 nondiabetic subjects and 397 type 2 diabetic patients, 192 nondiabetic subjects (M/F, 112:80; 52.1 $\pm$ 13.2 years) and 304 type 2 diabetic patients (M/F, 179:125; 53.4 $\pm$ 13.3 years) were finally included into the study.

Two experienced ophthalmologists performed fundus examination within 6 months of enrollment. If there was any suspicion about the presence of DR, the patient was further evaluated by fluorescein angiography. Type 2 diabetic patients were classified as follows: a non-DR (NDR) group (no evidence of retinal change due to diabetes), a nonproliferative DR (NPDR) group (existence of retinal hemorrhage and/or exudate but not new vessel formation), and a proliferative DR (PDR) group (presence of neovascularization). Diabetic macular edema was defined as thickening of the retina and/or hard exudates within a 1-disk diameter of the center of the macula according to the Early Treatment Diabetic Retinopathy

Study criteria [22]. In DME, the focal type was defined as a focal retinal thickening and intraretinal leakage from foci of microaneurysms in the macular area, and the diffuse type was defined as a retinal thickening including the entire macula with intraretinal leakage from dilated capillary. The ischemic type was defined as a macular ischemia observed through fluorescein angiography. All patients without DR did not have any DME.

Type 2 diabetic patients were treated with diet alone (125.5 kJ/kg [30 kcal/kg] standard body weight per day containing of 60% carbohydrate and 25% fat), with diet in combination with oral hypoglycemic agents (sulfonylurea, biguanide, α-glucosidase inhibitor, thiazolidinedione), or with diet in combination with insulin therapy.

2.2. Laboratory measurements

Fasting plasma glucose (FPG) (coefficient of variation [CV], 1.1%), HbA_{1c} (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 assays. Reference ranges of total cholesterol, triglyceride, and high-density lipoprotein cholesterol were 130 to 250, 40 to 194, and 35 to 71 mg/dL, respectively.

2.3. Determination of genotypes

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 a binding column tube and was centrifuged at 8000 rpm for 1 minute. Washing and elution were performed, and the genomic DNA was preserved at −70°C.

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

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

2.4. Statistical analysis

Data are expressed as the mean ± SD. Statistical analysis was performed using SPSS version 10.0 (SPSS, Chicago, IL). Comparisons of clinical and laboratory variables between nondiabetic subjects and type 2 diabetic patients were done by unpaired Student *t* test or χ^2 test as appropriate. Skewed data were logarithmically transformed before analysis. Genotypes and allele distributions of the Mn-SOD and ACE genes in all subjects were analyzed by χ^2 test. Basic characteristics, genotypes, and allele distributions of the Mn-SOD and ACE genes of groups according to the presence and severity of DR or DME were compared by analysis of variance. Clinical and laboratory parameters between the allele carriers and noncarriers were analyzed by unpaired Student *t* test or χ^2 test. Tests for Hardy-Weinberg equilibrium were performed using χ^2 test. A P value of less than .05 was considered significant. For determination of independent risk factors associated with DR or DME, multivariate analysis was conducted with multiple logistic regression, in which only variables showing a significant association with DR or DME in univariate analysis ($P < .05$) were introduced. Odds ratio (OR) and 95% confidence interval

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

Variable	Control subjects	Diabetic patients
n	192	304
Age (y)	52.1 ± 13.2	53.4 ± 13.3
Sex (M/F)	112/80	179/125
BMI (kg/m^2)	23.2 ± 2.7	23.6 ± 3.2
Hypertension (n)	61 (31.8)	104 (34.2)
SBP (mm Hg)	134 ± 17	138 ± 19
DBP (mm Hg)	91 ± 12	97 ± 14
FPG (mg/dL)	96 ± 8	147 ± 22*
HbA_{1c} (%)	5.6 ± 0.4	8.7 ± 1.1*
Total cholesterol (mg/dL)	206.3 ± 6.5	208.1 ± 7.2
Triglyceride (mg/dL)	203.4 ± 12.8	207.9 ± 13.6
HDL-C (mg/dL)	53.7 ± 5.4	51.8 ± 5.2
Serum creatinine (mg/dL)	0.7 ± 0.3	0.7 ± 0.4
Mn-SOD genotype		
VV (n)	152 (79.2)	243 (79.9)
VA + AA (n)	40 (20.8)	61 (20.1)
A allele	0.112	0.107
ACE genotype		
II (n)	71 (37.0)	114 (37.5)
ID + DD (n)	121 (63.0)	190 (62.5)
D allele	0.404	0.392

Data are expressed as mean ± SD. The numbers in parentheses are percentage. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol.

* $P < .05$ vs the group of control subjects.

Table 2
Clinical and laboratory characteristics in type 2 diabetic patients according to DR and DME

Variable	DR (−)	DR (+)				
		All	NPDR without DME	NPDR with DME	PDR without DME	PDR with DME
n	174	130	76	14	17	23
Age (y)	53.7 ± 12.9	53.1 ± 12.3	53.1 ± 11.0	53.2 ± 10.8	53.1 ± 10.4	54.0 ± 11.2
Sex (M/F)	102/72	77/53	45/31	8/6	11/6	13/10
Diabetic duration (y)	9.4 ± 2.8	11.4 ± 3.7*	10.3 ± 2.1	11.0 ± 2.4	11.9 ± 3.1	12.5 ± 3.3
BMI (kg/m^2)	23.6 ± 2.7	23.7 ± 3.0	23.7 ± 2.2	23.7 ± 2.6	23.8 ± 2.5	23.7 ± 2.1
Hypertension (n)	51 (29.3)	53 (40.8)*	26 (34.2)	6 (42.9)†	9 (52.9)	12 (52.2)
SBP (mm Hg)	136 ± 13	141 ± 16	138 ± 12	142 ± 11	143 ± 14	147 ± 12
DBP (mm Hg)	94 ± 11	99 ± 12	94 ± 8	97 ± 10	104 ± 9	109 ± 8
FPG (mg/dL)	143 ± 16	152 ± 18	147 ± 11	151 ± 12	156 ± 14	161 ± 13
HbA_{1c} (%)	8.5 ± 0.7	8.9 ± 0.9	8.5 ± 0.7	8.6 ± 0.6	8.8 ± 0.7	9.0 ± 0.7
Total cholesterol (mg/dL)	206.1 ± 5.3	212.3 ± 5.4	207.2 ± 4.4	209.1 ± 4.1	213.4 ± 4.2	218.6 ± 4.9
Triglyceride (mg/dL)	205.2 ± 11.7	208.6 ± 12.2	205.4 ± 9.6	208.3 ± 10.2	213.1 ± 10.7	217.8 ± 11.1
HDL-C (mg/dL)	53.1 ± 4.6	50.4 ± 3.1	52.9 ± 2.2	51.7 ± 2.1	54.2 ± 2.5	48.1 ± 2.4
Serum creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.2
Therapy (D/O/I)	32/129/13	17/91/22	12/58/6	2/8/4	1/11/5	2/14/7

Data are expressed as mean ± SD. The numbers in parentheses are percentage. D indicates diet alone; O, oral hypoglycemic agent; I, insulin.
* $P < .05$ vs DR (−) group.
† $P < .05$ vs NPDR without DME group.

(95% CI) were also estimated.

3. Results

3.1. Comparison of nondiabetic and type 2 diabetic subjects

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, systolic and diastolic blood pressures, serum lipid profile, and serum creatinine levels between the 2 groups. However, FPG and HbA_{1c} levels in type 2 diabetic patients were significantly higher than those in nondiabetic subjects ($P < .05$). The numbers taking 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor were not different between the 2 groups (nondiabetic vs diabetic, 32 [16.7%] vs 61[20.1%]). Mn-SOD and ACE genotype distributions were consistent with Hardy-Weinberg equilibrium ($P = .97$ and $P = .34$) and did not differ between the 2 groups.

When nondiabetic subjects were divided into the normotensive (n = 131) and hypertensive (n = 61) groups, Mn-SOD and ACE genotype distributions and allele frequencies in the normotensive group did not differ from those in the hypertensive group (normotensive vs hypertensive: VV/VA + AA, 104 [79.4%]/27 [20.6%] vs 48 [78.7%]/13 [21.3%]; V/A, 0.889/0.111 vs 0.885/0.115; II/ID + DD, 48 [36.6%]/83 [63.4%] vs 23 [37.7%]/38 [62.3%]; I/D, 0.599/0.401 vs 0.590/0.410). In type 2 diabetic patients, there was no difference in prevalences of DR and DME between well-controlled (n = 18) and not well-controlled (n = 286) groups classified by 7.0% of HbA_{1c} levels.

3.2. Comparison of DR and DME subgroups

As shown in Table 2, the patients with DR (DR [+] group, n = 130), the NPDR and PDR groups, had significantly longer diabetic duration and higher prevalence of hypertension than those patients without DR (DR [−] group, n = 174) ($P < .05$). Prevalence of hypertension and the number of patients on insulin therapy in the PDR group were significantly different from those in the NDR and NPDR groups (NDR/NPDR/PDR: hypertension, 51 [29.3%]/32 [35.6%]/21 [52.5%], $P < .05$; insulin therapy, 13 [7.5%]/10 [11.1%]/ 12 [30.0%], $P < .05$). In the NPDR group, prevalence of hypertension was significantly higher than in the NDR group ($P < .05$). The PDR group had

Table 3
Genotype distributions and allele frequencies of the Mn-SOD and ACE gene polymorphisms in type 2 diabetic patients according to DR and DME

		DR (−)	DR (+)				
			All	NPDR	PDR	DME (−)	DME (+)
n		174	130	90	40	93	37
Mn-SOD	VV (n)	138 (79.3)	105 (80.8)	73 (81.1)	32 (80.0)	73 (78.5)	32 (86.5)
	VA + AA (n)	36 (20.7)	25 (19.2)	17 (18.9)	8 (20.0)	20 (21.5)	5 (13.5)
	A allele	0.110	0.104	0.100	0.113	0.118	0.068*
ACE	DD (n)	67 (38.5)	47 (36.2)	33 (36.7)	14 (35.0)	33 (35.5)	14 (37.8)
	ID + DD (n)	107 (61.5)	83 (63.8)	57 (63.3)	26 (65.0)	60 (64.5)	23 (62.2)
	D allele	0.388	0.396	0.394	0.400	0.398	0.393

Data are expressed as mean ± SD. The numbers in parentheses are percentage.
* $P < .05$ vs DME (−) group.

Table 4
Genotype distributions and allele frequencies of the Mn-SOD and ACE gene polymorphisms according to each type of DME in type 2 diabetic patients

		Focal type			Diffuse type			Ischemic type		
		All	NPDR	PDR	All	NPDR	PDR	All	NPDR	PDR
n		8	7	1	23	6	17	6	1	5
Mn-SOD	VV (n)	5 (62.5)	4	1	18 (78.3)	4	14	6 (100.0)	1	5
	VA + AA (n)	3 (37.5)	3	0	5 (21.7)	2	3	0 (0.0)	0	0
	A allele	0.188			0.109			0		
ACE	II (n)	4 (75.0)	3	1	10 (43.5)	4	6	1 (16.7)	0	1
	ID + DD (n)	4 (25.0)	4	0	13 (56.5)	2	11	5 (83.3)	1	4
	D allele	0.375			0.413			0.667		

The numbers in parentheses are percentage.

significantly longer diabetic duration and higher serum total cholesterol level than the NDR group (NDR vs PDR: diabetic duration, 9.4 ± 2.8 vs 12.1 ± 3.4, $P < .05$; total cholesterol, 206.1 ± 5.3 vs 216.2 ± 5.1, $P < .05$).

Between the non-DME (DME [−] group, n = 93) and DME (DME [+] group, n = 37) groups, there was no difference in age, sex, BMI, prevalence of hypertension, systolic and diastolic blood pressures, FPG, HbA_{1c} level, serum lipid profile, and serum creatinine level. Mn-SOD genotype distribution of type 2 diabetic patients was in Hardy-Weinberg equilibrium ($P = .67$).

Diabetic retinopathy and DME of the insulin-treated group (n = 35) were significantly more prevalent than those in the oral agent-treated group (n = 220) (insulin-treated vs oral agent–treated: DR, 22 [62.9%] vs 91 [41.4%], $P < .05$; DME, 11 [31.4%] vs 22 [10.0%], $P < .05$).

3.3. Association of the Mn-SOD and ACE gene polymorphisms with DR and DME

The A allele frequency of the Mn-SOD gene and the D allele frequency of the ACE gene were not different between the DR (−) and DR (+) groups. The allele frequencies also did not differ according to the stage of retinopathy (Table 3).

In the DR (+) group, the A allele frequency was significantly lower in the DME (+) group than in the DME (−) group ($P < .05$). The D allele frequency did not differ between the DME (+) and DME (−) groups.

In the DME (+) group, focal, diffuse, and ischemic types were found in 8 patients (7 NPDR patients, 1 PDR patient), 23 patients (6 NPDR patients, 17 PDR patients), and 6 patients (1 NPDR patient, 5 PDR patients), respectively (Table 4).

3.4. Determination of risk factors associated with DR and DME

In type 2 diabetic patients, the NDR, NPDR, and PDR groups between the A allele carriers (n = 61) and noncarriers (n = 243) were as follows: A allele carriers vs A allele noncarriers: NDR/NPDR/PDR, 36 (59.0%)/17 (27.9%)/8 (13.1%) vs 138 (56.8%)/73 (30.0%)/32 (13.2%). Clinical and laboratory parameters of the A allele carriers were not different from those of the A allele noncarriers except in the prevalence of hypertension and DME ($P < .05$) (Table 5). All parameters including DME did not differ between the D allele carriers and noncarriers of the ACE gene. In multivariate analysis conducted with multiple logistic regression, hypertension (OR, 1.69; 95%

Table 5
Clinical and laboratory characteristics in type 2 diabetic patients according to Mn-SOD and ACE genotypes

Variable	Mn-SOD genotype		ACE genotype	
	VV	VA + AA	II	ID + DD
n	243	61	114	190
Age (y)	53.4 ± 13.1	53.3 ± 12.7	53.6 ± 12.5	53.4 ± 12.3
Sex (M/F)	142/101	37/24	67/47	112/78
Diabetic duration (y)	10.3 ± 2.9	10.5 ± 3.2	10.7 ± 2.8	10.4 ± 2.6
BMI (kg/m^2)	23.6 ± 3.0	23.7 ± 3.1	23.7 ± 3.1	23.6 ± 2.9
Hypertension (n)	88 (36.2)	16 (26.2)*	35 (30.7)	69 (36.3)
SBP (mm Hg)	139 ± 14	136 ± 12	137 ± 14	138 ± 16
DBP (mm Hg)	98 ± 11	95 ± 11	96 ± 11	98 ± 13
FPG (mg/dL)	147 ± 16	145 ± 13	146 ± 17	147 ± 19
HbA_{1c} (%)	8.7 ± 1.0	8.7 ± 0.9	8.7 ± 0.9	8.7 ± 0.8
Total cholesterol (mg/dL)	209.3 ± 6.7	207.8 ± 5.3	209.2 ± 6.7	207.6 ± 5.2
Triglyceride (mg/dL)	208.2 ± 11.6	207.4 ± 11.2	208.3 ± 11.4	207.8 ± 11.3
HDL-C (mg/dL)	51.3 ± 4.2	51.6 ± 4.4	51.2 ± 4.3	52.1 ± 4.6
Serum creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
DME (n)	32 (13.2)	5 (8.2)*	14 (12.3)	23 (12.1)

Data are expressed as mean ± SD. The numbers in parentheses are percentage.
* $P < .05$ vs VV genotype group.

CI, 1.54-3.36; P = .02), diabetic duration (OR, 1.02; 95% CI, 1.01-1.04; P = .03), and insulin therapy (OR, 1.48; 95% CI, 1.02-2.18; P = .02) were associated with the stage of retinopathy. The A allele of the Mn-SOD gene (OR, 2.13; 95% CI, 1.14-4.07; P = .01), hypertension (OR, 1.57; 95% CI, 1.32-2.39; P = .02), and insulin therapy (OR, 1.59; 95% CI, 1.02-2.02; P = .03) were associated with DME.

4. Discussion

Amino acid sequences of Mn-SOD among species are highly preserved, and their homologies between human and rodent, and between rat and mouse are 93% and 96%, respectively [14,23]. This conservation may be critical to maintain enzymatic function. The *Sod2* gene encoding of Mn-SOD consists of 5 exons interrupted by 4 introns with typical splice junctions [24]. Two sequential differences resulting in polymorphisms of the *Sod2* gene, V16A and I58T, are identified at the 16th and 58th amino acid positions [14]. Structurally altered Mn-SOD variant with reduced activity produces unfavorable influences by increased mitochondrial oxidative stress related to diabetes. In V16A polymorphism, valine-to-alanine substitution changes the 3-dimensional structure of the signal peptide [15,16], and thereby enzymatic content within mitochondria is increased in condition of the signal peptide with alanine [25]. Because oxidative stress is augmented in diabetes, it is essential to evaluate whether V16A polymorphism is related to intra-mitochondrial amounts of Mn-SOD influences on the development of diabetes and its microvascular complications.

In previous studies, Nomiyama et al [19] reported that the A allele frequency was not different between Japanese type 2 diabetic patients and nondiabetic subjects, and Chistyakov et al [20] demonstrated similar results in Russian type 1 diabetic patients, although the A allele frequency was lower in Japanese than Russian nondiabetic subjects (0.144 vs 0.659). In the present study, the A allele frequency between nondiabetic subjects and type 2 diabetic patients did not differ, and the A allele frequency of nondiabetic subjects was similar to that of Japanese healthy subjects reported by Nomiyama et al (0.112 vs 0.144). It suggests that V16A polymorphism is not related to development of diabetes in the Korean, Japanese, and Russian populations. Discrepancy of the A allele frequency among these studies may result from racial or geographical difference because the allele frequency is often influenced by characteristics of the study population. In the study by Van Landeghem et al [26], the A allele frequencies among ethnical populations were various, for example, the A allele frequency of the Chinese population was significantly lower than that of the European populations.

In type 2 diabetic patients, the A allele frequency was similar between the DR (−) and DR (+) groups and was not different in accordance with the stage of retinopathy. However, the A allele frequency of the DME (+) group was significantly lower than that of the DME (−) group, and the A allele was associated with lower prevalence of DME. The results are consistent with the previous data reported by Chistyakov et al although diabetic types differed, and these indicate that V16A polymorphism is a susceptible genetic factor for DME but not DR. It is unclear why DME but not DR shows an association with V16A polymorphism. One possible explanation is that V16A polymorphism may be related to alteration of vascular permeability rather than vascular occlusion. In this regard, Awata et al [10] demonstrated that polymorphisms of the eNOS gene were associated with DME but not DR. However, they did not explain the cause of difference.

Classification of DME into focal, diffuse, and ischemic types is valuable because of different therapeutic strategies. For example, focal type is treated by focal laser therapy, whereas diffuse type is managed by grid laser therapy and/or pars plana vitrectomy. In this study, the A allele frequency was decreased in order of focal, diffuse, ischemic types (0.188, 0.109, 0.0). However, we could not evaluate the association of each type with the A allele frequency because of the small numbers of each type. Therefore, in a further large-scale study, it should be investigated which type of DME is associated with V16A polymorphism.

The DR (+) group had longer diabetic duration and higher prevalence of hypertension than the DR (−) group. Analyzing clinical and laboratory parameters in each stage of DR, prevalence of hypertension and patients on insulin therapy of the PDR group were different from those in the NDR and NPDR groups. Diabetic duration and serum total cholesterol level were longer and higher in the PDR group than in the NDR group, and the NPDR group than the NDR group had a higher prevalence of hypertension. Multivariate analysis revealed that hypertension, diabetic duration, and insulin therapy were associated with the stage of retinopathy, suggesting that these components are risk factors related to progression of DR. Therefore, we may be able to prevent progression of DR in the DR (−) group with long diabetic duration by strict blood pressure control because retinopathy is associated with hypertension (OR, 1.69) rather than diabetic duration (OR, 1.02).

Hypertension was associated with DME, but the A allele frequency between the normotensive and hypertensive nondiabetic subjects did not differ. These results mean that the A allele itself is associated with DME independently of hypertension. Interestingly, insulin therapy was also associated with DR and DME. It is likely that insulin therapy reflects lower insulin-secreting capacity as well as previous poor glycemic control and may concern with exacerbation of blood-retinal barrier breakdown [27]. The HbA_{1c} level was unrelated to DR and DME, contradicting the result of the Diabetes Control and Complication Trial that intensive treatment of diabetes resulted in significant risk reduction in DME [28]. In our study, HbA_{1c} levels may not be regarded as an indicator of long-term glycemic control.

When we analyzed I/D polymorphism in Korean type 2 diabetic patients and nondiabetic subjects, the DD genotype and D allele frequency had no difference between the 2 groups in the present study (DD genotype, 15.8% vs 14.6%; D allele frequency, 0.392 vs 0.404) as well as in the previous study (DD genotype, 12.1% vs 10.1%; D allele frequency, 0.342 vs 0.343) [21]. Ohno et al [29] demonstrated that the DD genotype was not different between Japanese type 2 diabetic patients and healthy subjects (13% vs 11%). In contrast, Arzu et al [30] presented the result that DD genotype was significantly increased in type 2 diabetic patients compared with healthy subjects in the Turkish population (48% vs 27%). These results indicate that I/D polymorphism does not affect development of type 2 diabetes, at least, in the Korean population, and that the D allele frequency is similar within the Far East area.

Because I/D polymorphism influences plasma and cellular ACE levels [31-34] determining production of angiotensin II, a potent vasoconstrictor as well as an angiogenic factor in diabetic animal model [35,36], it may be related to the development of DR. However, previous studies showed controversial results in type 2 diabetic patients probably because of different races and criteria of DR [37]. In this study, there was no difference in the D allele frequency between the DR (−) and DR (+) groups, among the stages of DR and between the DME (−) and DME (+) groups. Therefore, in the Korean population, I/D polymorphism may not be associated with DR and DME reported by previous studies [31,32,38-41].

Glucose and lipid levels may influence clinical and laboratory parameters, especially the prevalences of diabetic retinal complications. However, in the present study, when type 2 diabetic patients were classified into well-controlled and not well-controlled groups by 7.0% of HbA_{1c}, there was no difference in prevalences of DR and DME between the 2 groups. Although the numbers taking HMG-CoA reductase inhibitor were not different between nondiabetic and diabetic subjects, it cannot be completely excluded that medication using an HMG-CoA reductase inhibitor may act as a confounding factor.

In our study, prevalence of hypertension did not differ between nondiabetic subjects and type 2 diabetic patients, although it is known to be usually higher in diabetic patients. It is likely to be related to relatively short-term duration of diabetes. Another limitation is that Mn-SOD and ACE activities were not measured. Moreover, no evidence has been reported that V16A polymorphism alters oxidative stress in diabetic patients. Therefore, the pathophysiologic role and clinical significance of these polymorphisms should be clarified.

Optical coherence tomography (OCT), retinal thickness analyzer, fluorescein angiography, streoscopic fundus photography, and contact lens biomicroscopy have been applied for diagnosis of DME. In particular, OCT is a noncontact and noninvasive imaging technique and produces cross-sectional images of the retina by projecting a pair of near-infrared light beams into the eye. It also can be used to measure retinal thickness. In diabetic patients, OCT is applied to diagnose DME and to follow up DME during treatment [42]. It offers an objective and intuitive basis for evaluating and comparing the efficacy of different therapeutic modalities. However, we diagnosed DME by using conventional methods according to the Early Treatment Diabetic Retinopathy Study criteria that are still applied in many studies because OCT was not available [22]. Therefore, our results should be confirmed in further studies by using more objective techniques such as OCT, although conventional methods should also be used when new techniques are not always popular.

In conclusion, our results suggest that V16A polymorphism of the Mn-SOD gene is not related to the development of diabetes and progression of DR, but is associated with DME in Korean type 2 diabetic patients. Polymorphism of the Mn-SOD gene may also help to diagnose and treat DR and DME in different diabetic populations. However, further studies are needed to confirm whether these data can be applied to other ethnical populations and to identify the other genetic factors that play a role in development of diabetic retinal complications.

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