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Promoter polymorphisms of the vascular endothelial growth factor gene are associated with metabolic syndrome susceptibility in Koreans

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Abstract. Vascular endothelial growth factor (VEGF) is a potent regulator of angiogenesis. Patients with metabolic syndrome (MetS) have elevated plasma VEGF levels. The aim of the present study was to investigate the association between promoter polymorphisms (the -2578C>A and -1154G>A) of the *VEGF* gene and MetS susceptibility. A total of 640 subjects were enrolled in the study including 320 patients with MetS and 320 healthy controls. Genotyping of the *VEGF* single nucleotide polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism analysis. The CA and AA genotypes of the -2578C>A polymorphism were associated with decreased risk of MetS (P=0.018, P=0.003, respectively). For the -1154G>A polymorphism, although the GA genotype was more significantly frequent in MetS patients (P=0.022), the AA genotype and recessive model (GG+GA vs. AA) were protective against MetS susceptibility (P=0.016, 0.007, respectively). The A-G haplotype frequency composed of the -2578C>A and -1154G>A polymorphisms also differed between the 2 groups (P=0.011). The presented data suggested that the A alleles and A-G haplotype of the *VEGF* -2578C>A and -1154G>A polymorphisms are associated with decreased MetS susceptibility. To the best of the authors' knowledge, the current study is the first to investigate the associations between the *VEGF* -2578C>A and -1154G>A polymorphisms and MetS patients. Further evaluation is necessary to explore the associations between the *VEGF* polymorphisms and MetS patients in larger samples of other ethnic or racial populations.

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Introduction

Vascular endothelial growth factor (VEGF) is a potent regulator of angiogenesis. Inter-individual variations of plasma VEGF levels are reported in healthy subjects (1). Plasma VEGF levels are upregulated under conditions of increased angiogenesis (2). Thus, VEGF has attracted considerable attention for its role in development of cancer vascular and metabolic diseases.

Clones of the human *VEGF* gene have been isolated and sequenced (3). It is located on chromosome 6p21.3 and is composed of 8 exons and 7 introns spanning approximately 14 kb. *VEGF* mRNA expression is rapidly and reversibly induced by hypoxia *in vitro* and *in vivo* (4). So far, a number of polymorphic sites have been identified in the *VEGF* gene.

Sequence variations of *VEGF* polymorphisms can influence plasma VEGF levels (5,6). Among these, healthy subjects with the A alleles of the -2578C>A and -1154G>A polymorphisms in the promoter region have reduced plasma VEGF levels, when compared with other allele carriers. The CC homozygotes of the -634C>G polymorphism in the 5'-untranslated region (UTR) have significantly higher VEGF levels than other genotypes (7). In the 936C>T polymorphism of the 3'-UTR, the 936T allele is associated with lower VEGF plasma levels than in non-carriers (8,9).

According to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) criteria, the metabolic syndrome (MetS) can be diagnosed based on abdominal obesity, dyslipidemia, hypertension and hyperglyceridemia (10). Development of MetS may be caused by interaction between the genes and environment. It markedly increases the risk of developing cardiovascular diseases, as well as type 2 diabetes (11,12). MetS is prevalent in 25-35% of adults in advanced countries (13).

Elevated plasma VEGF levels reportedly occur in patients with MetS (14). The components of MetS, such as body mass index (BMI), waist circumference (WC), blood pressure and inflammation are significantly correlated with plasma VEGF levels (15,16). The role of genetic factors in susceptibility to MetS is unclear. *VEGF* polymorphisms are associated with many kinds of diseases, yet the results are inconsistent to date. However, association studies between *VEGF* polymorphisms

and patients with MetS are very rare. Therefore, the present study investigated the possible associations of the -2578C>A and -1154G>A polymorphisms in the promoter region of the *VEGF* gene with MetS patients.

Materials and methods

Study subjects. A total of 640 subjects enrolled in the study included 320 MetS patients and 320 healthy controls. The diagnostic criteria of MetS patients based on slight modifications of NCEP-ATP III definition were previously described in detail (10). The control group consisted of healthy subjects without other vascular or metabolic diseases by health screening test. Comparisons of the demographic characteristics between the controls and MetS patients have been suggested in a previous study of the authors (17). The MetS patients and control subjects presented no differences in age. Except for significantly lower levels of high density lipoprotein-cholesterol in patients than in controls, all the conventional risk factors including BMI, triglycerides, systolic blood pressure, diastolic blood pressure and WC were significantly higher in MetS patients than in controls. The blood samples were obtained from the Biobank of Jeju National University Hospital (Jeju, South Korea) supported by the Ministry of Health and Welfare (Sejong, South Korea). Written informed consent was obtained from all study subjects. The experimental design was approved by the Institutional Review Board of Jeju National University Hospital (Jeju, South Korea).

Genotyping of the VEGF polymorphisms. DNA isolated by salt extraction was genotyped by the conventional polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism technique. The primers and reaction conditions for PCR were as described previously (18). Following amplification, the PCR products were digested with restriction enzymes, *Ava*II and *Mnl*I (New England Biolabs, Inc., Ipswich, MA, USA), for genotyping of the -2578C>A and -1154G>A polymorphisms, respectively.

Statistical analysis. Clinical profiles of the MetS patients and controls were compared by Student's t-test. The Chi-squared test was used to compare genotype and allele frequencies, and to determine deviations from the Hardy-Weinberg equilibrium. Multivariate logistic regression analysis was performed to derive the odd ratios (OR). Statistical analyses were done with StatsDirect Statistical software (version 2.4.4; StatsDirect Ltd, Altrincham, UK). $P<0.05$ was considered to indicate a statistically significant difference.

Results

The genotype frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms are presented in Table I. The allelic frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms presented no deviation from the Hardy-Weinberg equilibrium in MetS patients and healthy controls.

For the -2578C>A polymorphism, the CC and AA genotypes were associated with decreased risk for MetS (P=0.018, P=0.003, respectively). In addition, the dominant (CC vs. CA+AA) or recessive (AA+CA vs. AA) models demonstrated the same trend (P=0.003, P=0.009). On the other hand, the AA genotype and recessive model (GG+GA vs. AA) of the -1154G>A polymorphism were found less frequently in patients with MetS, as compared with controls (P=0.016, 0.007, respectively), although the GA genotype was significantly more frequent in MetS patients (P=0.022). When the data of the *VEGF* -2578C>A and -1154G>A polymorphisms were stratified by sex (Table II), the association was sex-specific. In the male group, the dominant model of the -2578C>A polymorphism and the GA genotype and recessive model of the -1154G>A polymorphism had significant differences in distributions between the two groups.

The haplotype distributions composed of 2 polymorphisms differed between the 2 groups (Table III). The A-G haplotype of the -2578C>A and -1154G>A polymorphisms were less frequent in patients with MetS, as compared with controls (P=0.011). When the data of the haplotype were stratified by sex, the A-G haplotype of the female group demonstrated the same pattern with total subjects. However, the C-A haplotype significantly increased the risk of MetS (P=0.030) in the male group.

Discussion

Plasma VEGF levels differ among individuals (1). *VEGF* mRNA expression is elevated in both physiological and pathological states with increased angiogenesis (2). Patients with MetS have an associated increase in plasma VEGF levels (14). It is reasonable to hypothesize that VEGF is a likely risk factor for developing MetS. However, there are no known reports on the association between *VEGF* -2578C>A and -1154G>A polymorphisms and MetS susceptibility. Therefore, the authors investigated the effects of the -2578C>A and -1154G>A VEGF gene promoter polymorphisms on MetS susceptibility in the Korean population.

The AA genotypes for the *VEGF* -2578C>A and -1154G>A polymorphisms were associated with reduced VEGF expression in mononuclear cells from peripheral blood (5). In the present study, the AA and CA genotypes of the -2578C>A polymorphism and the AA genotype and recessive model (GG+GA vs. AA) of the -1154G>A polymorphism were identified less frequently in patients with MetS, as compared with controls. Thus, the *VEGF* -2578C>A and -1154G>A polymorphisms may contribute to differences in susceptibility to and severity of MetS between individuals. In particular, the association was male-specific, suggesting that VEGF production according to *VEGF* -2578C>A and -1154G>A genotypes may differ between men and women. For example, Fernández-Santiago *et al* (19) reported that the *VEGF* -1154G>A polymorphism is associated with affection status in women with amyotrophic lateral sclerosis of several European populations, suggesting that the VEGF effect may be dependent on the sex ratio of the sample.

The -2578C>A polymorphism is in complete linkage with deletion/insertion of an 18 bp fragment in the -2549 region of the *VEGF* gene promoter. An 18 bp deletion construct presented a 1.95-fold increase in transactivation by link with the C allele (20). The *VEGF* -2578C>A polymorphic site is located in a potential binding site of the Myeloid Zinc Finger-1 (MZF-1) transcription factor. Thus the *VEGF* -2578C>A polymorphism may influence promoter activity and variations of

Table I. Comparison of the genotype frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms between control subjects and patients with MetS.

Genotype	Controls (n=320)	MetS (n=320)	AOR (95% CI)	P-value[a]
VEGF -2578C>A				
CC	164 (51.3)	195 (60.9)	1.000 (reference)	
CA	134 (41.9)	118 (36.9)	0.661 (0.468-0.932)	0.018
AA	22 (6.9)	7 (2.2)	0.248 (0.099-0.618)	0.003
CC vs. CA+AA			0.601 (0.430-0.841)	0.003
CC+CA vs. AA			0.300 (0.122-0.738)	0.009
VEGF -1154G>A				
GG	200 (62.5)	181 (56.6)	1.000 (reference)	
GA	104 (32.5)	135 (42.2)	1.498 (1.059-2.118)	0.022
AA	16 (5.0)	4 (1.3)	0.246 (0.079-0.769)	0.016
GG vs. GA+AA			1.314 (0.939-1.838)	0.112
GG+GA vs. AA			0.207 (0.066-0.645)	0.007

[a]Adjusted by age and sex. *VEGF*, vascular endothelial growth factor; AOR, adjusted odds ratio; CI, confidence interval; MetS, metabolic syndrome.

VEGF protein levels among individuals. Although there is no report on the association between the -2578C>A polymorphism and MetS susceptibility, Liu *et al* (21) have reported that the -2578A allele is protective against the development of endometriosis in North Chinese women. In contrast, Maltese *et al* (22) reported that the AA homozygote of the -2578C>A polymorphism had increased susceptibility to colorectal cancer in the Italian population. Howell *et al* (23) have reported that the AA genotype was a risk factor and CC was protective for atherosclerosis development in the English population.

The *VEGF* -1154G>A polymorphic locus results in a substitution of Pax2 or Sp1 binding site by -1154A in place of the predicted MZF-1 binding site (24). Therefore, the sequence change at the -1154G>A polymorphic site may affect the transcription level and thus regulate VEGF protein production between individuals. The -1154AA genotype is associated with a decreased prostate cancer risk and less advanced melanomas in an English population (25,26). Liu *et al* (21) also reported that the -1154A allele is protective against the development of endometriosis in North Chinese women. In contrast, the -1154A allele is associated with an increased risk for recurrent spontaneous abortion in a Greek population (27); and increased *VEGF* expression in patients of ovarian or breast cancer in Austrian (28) and American populations (29), respectively. On the other hand, Sfar *et al* (30) reported that the GG and GA genotypes are linked with a high risk of developing prostate cancer in the Tunisian population. An increased association between non-small cell lung cancer and the -1154 G allele was reported in a Greek population (31).

In the present study, the A-G haplotype distribution comprising the *VEGF* -2578C>A and -1154G>A polymorphisms differed between the patients with MetS and controls. Although association between the haplotypes of the *VEGF* -2578C>A and -1154G>A polymorphisms and MetS patients has not yet been evaluated, several studies have reported that haplotypes of the *VEGF* polymorphisms may contribute to the susceptibility of individuals and the severity of a specific disease. For example, Liu *et al* (21) reported that the C-G-T, A-A-C, A-A-T and C-A-T haplotypes of the *VEGF* -2578C>A, -1154G>A and -460C>T polymorphisms are associated with endometriosis in North Chinese women. The A-A-G and A-G-G haplotypes of the -2578C>A, -1154G>A and -634G>C polymorphisms are associated with decreased *VEGF* gene transcription and lower plasma VEGF levels in patients with amyotrophic lateral sclerosis from Sweden, Belgium and England (32), less advanced melanoma in the English population (26), and the risk of acute cerebral infarction in the Chinese population (33).

To date, *VEGF* polymorphisms associated with various disorders such as cancers, metabolic or vascular diseases have attracted considerable attention. However, they are not consistent according to the ethnicities studied. The *VEGF* -2578C>A and -1154G>A polymorphisms are associated with either risk or protective effect for diseases in the various populations studied. This discordance may be due to differences in the genetic background of studied populations. The genotype and allele frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms may differ among populations. For example, the *VEGF* -2578C>A and -1154G>A polymorphism data differ among healthy populations (34-37). The reported allele frequency of the *VEGF* -2578A allele was higher in white people (0.378 to 0.504) than in Brazilian black people (0.290) and Asians (0.276 to 0.280), including Chinese and Korean populations. Regarding the -1154G>A polymorphism, the frequency of the *VEGF* -1154A allele (0.299 to 0.347 in white people vs. 0.160 in Brazilian black people and 0.173 to 0.131 in Asians) also indicates ethnic differences, similar to the *VEGF* -2578A allele. Thus, the allele frequencies of the -2578C>A and -1154G>A polymorphisms present ethnic or racial differences, indicating ethnic or racial disparities in the susceptibility to diseases.

Table II. The genotype frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms according to sex.

Genotype	Male				Female			
	Controls (n=152)	MetS (n=254)	AOR (95% CI)	P-value[a]	Controls (n=168)	MetS (n=66)	AOR (95% CI)	P-value[a]
VEGF -2578C>A								
CC	73 (48.0)	149 (58.7)	1.000 (reference)		91 (54.2)	46 (69.7)	1.000 (reference)	
CA	70 (46.1)	98 (38.6)	0.670 (0.440-1.019)	0.061	64 (38.1)	20 (30.3)	0.634 (0.340-1.182)	0.152
AA	9 (5.9)	7 (2.8)	0.373 (0.131-1.061)	0.065	13 (7.7)	0 (0.0)	N/A	N/A
CC vs. CA+AA			0.637 (0.423-0.959)	0.031			0.547 (0.295-1.014)	0.055
CC+CA vs. AA			0.448 (0.161-1.246)	0.124			N/A	N/A
VEGF -1154G>A								
GG	99 (65.1)	142 (55.9)	1.000 (reference)		101 (60.1)	39 (59.1)	1.000 (reference)	
GA	44 (28.9)	108 (42.5)	1.679 (1.084-2.601)	0.020	60 (35.7)	27 (40.9)	0.985 (0.536-1.812)	0.962
AA	9 (5.9)	4 (1.6)	0.321 (0.095-1.082)	0.067	7 (4.2)	0 (0.0)	N/A	N/A
GG vs. GA+AA			1.450 (0.953-2.204)	0.083			0.894 (0.490-1.631)	0.714
GG+GA vs. AA			0.265 (0.080-0.883)	0.031			N/A	N/A

[a]Adjusted by age and sex. *VEGF*, vascular endothelial growth factor; AOR, adjusted odds ratio; CI, confidence interval; MetS, metabolic syndrome.

Table III. Haplotype frequencies of the *VEGF* -2578C>A and -1154G>A polymorphisms.

Haplotype	Controls (2n=640)	MetS (2n=640)	OR (95% CI)	P-value[a]
-2578C>A/-1154G>A				
Overall				
C-G	388 (60.7)	419 (65.5)	1.080 (0.905-1.288)	0.418
C-A	74 (11.5)	89 (13.9)	1.203 (0.867-1.668)	0.280
A-G	116 (18.1)	78 (12.1)	0.672 (0.495-0.914)	0.011
A-A	62 (9.7)	54 (8.5)	0.871 (0.595-1.275)	0.498
Male	(2n=304)	(2n=480)		
C-G	192 (63.2)	327 (64.3)	1.019 (0.811-1.280)	0.908
C-A	24 (7.9)	69 (13.6)	1.720 (1.058-2.797)	0.030
A-G	50 (16.4)	65 (12.8)	0.778 (0.524-1.155)	0.220
A-A	38 (12.5)	47 (9.2)	0.740 (0.472-1.162)	0.198
Female	(2n=336)	(2n=160)		
C-G	197 (58.5)	92 (69.9)	1.189 (0.864-1.636)	0.288
C-A	49 (14.7)	20 (14.9)	1.039 (0.595-1.815)	0.887
A-G	65 (19.5)	13 (9.6)	0.509 (0.272-0.955)	0.037
A-A	25 (7.3)	7 (5.5)	0.713 (0.301-1.688)	0.543

[a]Fisher's exact test. *VEGF*, vascular endothelial growth factor; OR; odds ratio, CI, confidence interval; MetS, metabolic syndrome.

In conclusion, the study results suggested that the A alleles and A-G haplotype of the *VEGF* -2578C>A and -1154G>A polymorphisms were protective against MetS susceptibility in the Korean population. However, to the best of the authors' knowledge, there is no report on association between the *VEGF* -2578C>A and -1154G>A polymorphisms and patients with MetS. Therefore, the results need to be confirmed in sufficiently large homogeneous populations of different racial or ethnic groups.

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