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Adiponectin gene polymorphism 45T>G is associated with carotid artery plaques in patients with type 2 diabetes mellitus

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Abstract

Adiponectin has been reported to have a wide range of antiatherogenic actions. Two common single nucleotide polymorphisms (SNPs) at the adiponectin locus (45T>G and 276G>T) have been reported to be associated with diabetes and cardiovascular diseases. The aim of this study was to examine the association between common polymorphisms of the adiponectin gene (*ACDC*) and carotid atherosclerosis in patients with type 2 diabetes mellitus. A total of 708 unrelated patients with type 2 diabetes mellitus were recruited. SNP45 and SNP276 *ACDC* were genotyped, and B-mode ultrasonography of the carotid arteries was performed to measure carotid intima-media thickness and assess the presence of carotid artery plaques (CAP). Although there was no significant difference in carotid intima-media thickness according to *ACDC* genotype, subjects carrying the SNP45 GG genotype had a significantly higher risk of having CAP (odds ratio, 2.468; $P = .045$) compared with carriers of the T allele after adjustment for possible confounding factors. This study suggests that the GG genotype at *ACDC* SNP45 is associated with the presence of CAP and may contribute to atherosclerosis in type 2 diabetes mellitus.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in patients with diabetes. Compared with nondiabetic individuals, diabetic patients have a 3-fold higher risk of developing atherosclerosis and its clinical complications [1]. Both coronary artery disease and type 2 diabetes mellitus have a strong genetic basis [2]. They often occur together, and epidemiologic evidence suggests that this occurrence also has a genetic basis [3].

Adiponectin is an adipocytokine that plays pivotal roles in the regulation of insulin action and the metabolism of glucose and lipids [4,5]. It also performs many protective actions in the prevention of the initiation and progression of atherosclerosis by means of direct anti-inflammatory and antiatherogenic effects [6-8]. A strong linkage has been reported between the chromosomal region encompassing the adiponectin gene (*ACDC*) and cardiovascular risk factors [9,10]. Two single nucleotide polymorphisms (SNPs) at the adiponectin locus, +45 and +276, have recently been reported to be associated with diabetes and coronary heart disease [11-15].

Carotid atherosclerosis, which can be noninvasively measured by assessment of carotid intima-media thickness (IMT) and carotid artery plaques (CAP), is known to be associated with an increased risk of CVDs, including stroke and myocardial infarction [16-19]. The association between the *ACDC* polymorphisms and carotid atherosclerosis, which can be an early marker of established clinical CVD, in subjects with type 2 diabetes mellitus is unclear.

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Therefore, this study aimed to examine the association between SNP45 and SNP276 of the adiponectin gene and carotid atherosclerosis, measured by carotid IMT and the presence of CAP, in patients with type 2 diabetes mellitus.

2. Materials and methods

2.1. Study subjects

A total of 708 unrelated Korean patients with type 2 diabetes mellitus were recruited from the outpatient clinic of the Severance Hospital Diabetes Center, Yonsei University Medical Center, from June 2003 to September 2005. Diabetes was defined according to the 1997 American Diabetes Association diagnostic criteria [20]. Exclusion criteria were as follows: type 1 diabetes mellitus, current malignancy, posttransplantation status, history of ketoacidosis, positive for glutamic acid decarboxylase (GAD) antibody, severe kidney or liver disease, a recent ischemic event, thyroid function abnormalities, or corticosteroid use. The study protocol was approved by the Institutional Review Board of the Yonsei University College of Medicine. All patients gave informed consent to participate in this study.

2.2. Genetic analysis

Genotyping of *ACDC* SNPs +45 and +276 was performed as previously reported [21,22]. Genomic DNA was extracted from leukocytes in whole blood [21]. The genotyping was analyzed by a single base primer extension assay using a SNaPShot assay kit according to the manufacturer's recommendations (ABI, Foster City, CA), as previously described [22]. The primer sequences used are shown in Table 1. Results were analyzed using the Gene Mapper software (ABI).

2.3. Clinical and biochemical measurements

Fasting blood samples were obtained from the subjects and stored at −70°C for subsequent assays. Low-density lipoprotein (LDL) cholesterol levels were calculated using the Friedewald formula [23]. High-sensitivity C-reactive protein (hsCRP) was measured as previously described [24]. Plasma glucose was measured using a glucose oxidase method. Hemoglobin A_{1c} (HbA_{1c}) values were determined by high-performance liquid chromatography. Insulin concentrations were measured using a radioimmunoassay kit (IRMA kit; DAINABOT, Tokyo, Japan). Plasma adiponectin concentrations were measured using commercial sandwich enzyme-linked immunosorbent assay kits (B-Bridge International, San Jose, CA). Intraassay and interassay coefficients of variation were 3.3% and 7.4%, respectively.

Table 1
Primers and probe sequences for the amplification of ACDC SNPs

SNP		Primers and probes
45T/G	Sense	TGGACGGAGTCCTTTGTAGG
	Antisense	AGATGCAGCAAAGCCAAAGT
	Probe	TTCTACTGCTATTAGCTCTGCCCGG
276G/T	Sense	CAGGAAACCACGACTCAAG
	Antisense	GGGTGAAATGGAAGTTAAGC
	Probe	TCTAGGCCTTAGTTAATAATGAATG

2.4. Carotid B-mode ultrasound measurement

Ultrasonography of the carotid artery was conducted bilaterally by high-resolution B-mode ultrasonography (LOGIQ9; GE Medical Systems, Milwaukee, WI) with a 10-MHz linear transducer, as previously described [25,26]. A sonographer who was unaware of the subjects' characteristics scanned both common carotid arteries (CCAs), the carotid bulb, and the proximal portion of the internal and external carotid arteries. Computer-assisted acquisition, processing, storage of B-mode images, and calculation of IMT were performed with the Intima Scope software (MediaCross, Tokyo, Japan) as previously described [26,27]. Carotid artery plaques were defined as a focal thickening >50% of the surrounding wall [28]. The intraobserver coefficient of variance was 2.1%.

2.5. Statistical analysis

Data are shown as means ± standard deviations. All calculations and statistical analyses were performed using the SAS Genetics software package version 9.1 (SAS Institute, Cary, NC). The allelic distribution was verified by Hardy-Weinberg equilibrium. The differences among genotypes were evaluated using a 1-way analysis of variance test, *t* test, or χ^2 test as appropriate. Multiple linear regression was used to assess differences in mean CCA IMT between the genotypes after adjustment for other factors. Differences in genotype distribution between subjects with and without CAP were assessed by the χ^2 test. Genotype-associated risk of having CAP under a dominant, recessive, and multiplicative inheritance model after adjusting for possible confounding factors was assessed using logistic regression methods. *P* values were adjusted for multiple comparison using the Bonferroni method. A P value $< .05$ was considered to be statistically significant.

3. Results

3.1. Subject characteristics

The clinical and biochemical characteristics of the subjects according to the adiponectin genotype at positions 45 and 276 are shown in Table 2. Overall, the subjects had a mean age of 61.9 years, a mean diabetes duration of 11.8 years, a mean body mass index (BMI) of 24.7 kg/m^2, and a mean HbA_{1c} of 7.4%. Among the study subjects, the allele frequencies of both loci did not significantly deviate from Hardy-Weinberg equilibrium. Subjects with the SNP276 TT genotype had a lower fasting plasma glucose level than those with the GG genotype ($P = .019$). Otherwise, there were no significant differences in terms of

Table 2
Clinical and biochemical characteristics of the subjects according to adiponectin genotype at positions 45 and 276

	SNP45			*P*	SNP276			*P*
	TT	TG	GG		GG	GT	TT	
n (male/female)	346 (146/200)	306 (133/173)	56 (28/28)	.55	351 (142/209)	297 (133/164)	60 (32/28)	.14
Age (y)	62.1 ± 10.2	61.8 ± 9.3	60.9 ± 8.2	.68	61.7 ± 9.3	62.0 ± 10.1	62.3 ± 9.7	.89
Duration of diabetes (y)	11.7 ± 7.6	12.0 ± 6.7	11.5 ± 6.7	.79	11.8 ± 6.8	11.8 ± 7.3	11.5 ± 8.2	.93
Current smoking (%)	10.8	11.7	4.3	.31	11.0	11.0	7.5	.74
Body weight (kg)	63.2 ± 10.5	64 ± 10.2	63.3 ± 10.4	.61	63.5 ± 10.5	63.4 ± 10.0	64.8 ± 11.2	.64
BMI (kg/m^2)	24.7 ± 3.3	24.7 ± 3.3	24.6 ± 2.9	.96	24.8 ± 3.3	24.6 ± 3.1	25.1 ± 3.3	.45
Systolic BP (mm Hg)	127.1 ± 14	127.3 ± 16.1	128.3 ± 11.6	.87	127.7 ± 16.1	127.1 ± 13.6	126.0 ± 12.2	.67
Diastolic BP (mm Hg)	78.4 ± 7.3	78.6 ± 7.3	78.6 ± 6.5	.91	78.7 ± 7.2	78.2 ± 7.3	78.6 ± 6.7	.73
FPG (mmol/L)	7.14 ± 1.99	7.44 ± 2.07	7.49 ± 2.23	.15	7.44 ± 2.11	7.26 ± 0.40	6.65 ± 1.60	.02
2-h PPG (mmol/L)	11.30 ± 3.94	11.18 ± 3.96	12.43 ± 5.41	.11	11.53 ± 4.32	11.27 ± 3.84	10.47 ± 3.85	.17
HbA_{1c} (%)	7.3 ± 1.2	7.4 ± 1.2	7.5 ± 1.1	.51	7.4 ± 1.2	7.3 ± 1.2	7.2 ± 1.0	.24
Adiponectin (μg/mL)	9.83 ± 8.50	10.21 ± 8.81	10.52 ± 11.5	.87	9.61 ± 8.33	10.73 ± 9.55	9.31 ± 8.63	.31
Total cholesterol (mmol/L)	4.62 ± 0.89	4.63 ± 0.81	4.59 ± 0.85	.96	4.65 ± 0.88	4.61 ± 0.83	4.50 ± 0.80	.41
Triglyceride (mmol/L)	1.70 ± 1.13	1.68 ± 1.07	1.89 ± 1.13	.33	1.78 ± 1.20	1.62 ± 1.00	1.68 ± 0.98	.23
HDL cholesterol (mmol/L)	1.31 ± 0.30	1.32 ± 0.30	1.31 ± 0.39	.84	1.32 ± 0.31	1.32 ± 0.30	1.23 ± 0.28	.07
LDL cholesterol (mmol/L)	2.54 ± 0.82	2.54 ± 0.75	2.42 ± 0.72	.57	2.52 ± 0.81	2.55 ± 0.74	2.50 ± 0.83	.89
Antiplatelet agent use (%)	39.8	37.7	35.7	.78	39.4	36.9	41.7	.71
Mean CCA IMT (mm)	0.741 ± 0.164	0.766 ± 0.170	0.769 ± 0.170	.12	0.751 ± 0.164	0.758 ± 0.176	0.753 ± 0.144	.87
Presence of CAP (%)	53.5	56.9	67.9	.12	59.5	53.2	50.0	.16
History of CVD (%)	17.1	16.4	8.9	.30	15.8	16.2	18.3	.88

Data are means ± SD, unless otherwise indicated. BP indicates blood pressure; FPG, fasting plasma glucose.

age, duration of diabetes, BMI, HbA_{1c}, hsCRP, serum adiponectin, or lipid profiles between the SNP45 and SNP276 genotypes (Table 2). There was also no difference in medication for diabetes or hypertension according to the SNP45 and SNP276 genotypes.

3.2. Adiponectin gene polymorphisms and mean CCA IMT

Carriers of the allele +45G had significantly higher mean CCA IMT than those with the major allele homozygote genotype (TG + GG, 0.767 ± 0.170 mm vs TT, 0.741 ± 0.164 mm; P = .032) after adjustment for age, sex, and BMI. However, the significance was attenuated after adjusting for other risk factors (data not shown). There was no significant difference in the mean CCA IMT according to the SNP276 genotype (Table 2).

3.3. Adiponectin gene polymorphisms and CAP

The clinical and biochemical characteristics of subjects with and without CAP are shown in Table 3. Compared with patients without CAP, patients with CAP were older and had a longer duration of diabetes, higher systolic blood pressure, higher 2-hour postprandial plasma glucose (PPG) levels, higher mean CCA IMT, and lower HDL cholesterol levels. Individuals with CAP also showed a higher prevalence of coronary artery disease and stroke (Table 3).

After adjustments for age, sex, BMI, duration of diabetes, HDL cholesterol level, 2-hour PPG, current smoking status, and systolic blood pressure, subjects carrying the SNP45 GG genotype had a significantly higher risk of having CAP (odds ratio [OR], 2.468; 95% confidence interval [CI], 1.195-5.098; P = .045) compared with carriers of the T allele. There was no significant difference in the risk for CAP for SNP276 after adjustment for possible confounding factors (Table 4).

4. Discussion

In Korean patients with type 2 diabetes mellitus, who have a relatively homogenous ethnic background, we assessed the association of 2 common polymorphisms of the adiponectin gene and carotid atherosclerosis using mean CCA IMT and the presence of CAP as measurements of carotid atherosclerosis. There was no significant difference in CCA IMT according to the different genotypes of SNP45 and SNP276. However, SNP45 T>G was associated with a higher risk of having CAP. Although there was a trend toward having a lower risk of CAP in SNP276 T allele carriers, this was not statistically significant after adjustment for other factors. To our knowledge, this report is the first to show a significant association between adiponectin gene polymorphisms and CAP.

SNP45 and SNP276 of the adiponectin gene are common SNPs (frequency >20%) in Korean patients with type 2 diabetes mellitus and have been reported to be associated with obesity, insulin resistance, type 2 diabetes mellitus [11,12], and CVDs [13-15,29], although the mechanism of how these polymorphisms alter the action of adiponectin is not yet fully elucidated. Because there is a close association with carotid atherosclerosis and cardiovascular end points [16-19,30], we evaluated whether these polymorphisms also had influences on ultrasound-defined carotid atherosclerosis. Carotid IMT and CAP may be a more proximal phenotype in the pathway from DNA sequence variation to CVD, which is

Table 3
Baseline characteristics of individuals with and without CAP

	With carotid plaque	Without carotid plaque	*P*
n (male/female)	397 (174/223)	311 (133/178)	.78
Age (y)	64.8 ± 8.2	58.2 ± 10.2	<.001
Duration of diabetes (y)	13.0 ± 7.5	10.2 ± 6.3	<.001
Current smokers (%)	10.3%	11.1%	.74
Weight (kg)	63.7 ± 10.6	63.4 ± 10.1	.64
BMI(kg/m^2)	24.8 ± 3.4	24.6 ± 3.1	.26
Systolic BP (mm Hg)	128.8 ± 15.4	125.4 ± 13.7	.003
Diastolic BP (mm Hg)	78.6 ± 7.3	78.3 ± 7.0	.56
FPG (mmol/L)	7.20 ± 1.95	7.43 ± 2.16	.15
2-h PPG (mmol/L)	11.63 ± 4.19	10.96 ± 3.93	.03
HbA_{1c} (%)	7.3 ± 1.2	7.4 ± 1.2	.58
Adiponectin (μg/mL)	10.46 ± 9.25	9.37 ± 8.21	.26
hsCRP (mg/L)	1.24 ± 1.66	1.26 ± 1.77	.31
Total cholesterol (mmol/L)	4.60 ± 0.89	4.65 ± 0.80	.52
HDL cholesterol (mmol/L)	1.29 ± 0.30	1.34 ± 0.31	.02
Triglyceride (mmol/L)	1.74 ± 1.17	1.65 ± 1.01	.51
LDL cholesterol (mmol/L)	2.52 ± 0.82	2.54 ± 0.74	.65
Mean CCA IMT (mm)	0.795 ± 0.173	0.701 ± 0.145	<.001
Insulin therapy (%)	16.2	14.3	.48
Antidyslipidemic therapy (%)	27.8	21.7	.06
Antiplatelet therapy (%)	54.3	18.4	<.001
Coronary artery disease (%)	10.8	2.9	<.001
History of stroke (%)	12.3	4.5	<.001

Data are means ± SD, unless otherwise indicated.

the overt clinical disease [31]. Thus, understanding the genetic contribution of adiponectin to carotid atherosclerosis should be of great importance.

Interestingly, our study shows that SNP45 T>G is associated with the presence of CAP. There was no significant difference in carotid IMT according to *ACDC* genotype. Mackevics et al [32] previously reported that there was no significant association between these SNPs and carotid IMT in healthy subjects. Carotid IMT and CAP may represent different stages of the atherosclerotic process. Intima-media thickness likely reflects earlier stages of atherogenesis, notably a hypertrophic response of arterial intimal and medial cells to lipid infiltration or hypertension [33]. In contrast, formed arterial plaques probably represent a later stage of atherogenesis related to inflammation, oxidation, endothelial dysfunction, and/or smooth muscle cell proliferation [34]. Adiponectin has been reported to modulate the inflammatory response of endothelial cells to oxidized LDL and the activation of monocytes and macrophages by inhibiting tumor necrosis factor α–induced monocyte adhesion and the expression of endothelial leukocyte adhesion molecule 1, vascular cell adhesion molecule 1, and intracellular adhesion molecule 1 in endothelial cells [6]. Adiponectin also inhibits proliferation and migration of smooth muscle cells [35] and stimulates the production of nitric oxide in endothelial cells [8]. These effects of adiponectin may play a significant role in attenuating the formation of arterial plaques. The adiponectin gene polymorphisms as shown in this study seem to play a role in plaque formation.

In the present study, carotid atherosclerosis was significantly associated with SNP45 only. Considering that SNP45 is a silent mutation for Gly 15, there is a possibility this SNP inactivates the gene by influencing pre–messenger RNA splicing or messenger RNA stability. Otherwise, it could be related to another functional locus not yet identified via linkage disequilibrium [36]. Further studies, such as those using a TagSNP approach, will aid in elucidating the mechanism of this association. Although several studies have shown the SNP276 G allele to be associated with CVDs, we could not find any significant association with carotid atherosclerosis. This may reflect the difference in genetic determinants between CVD and carotid atherosclerosis, or an ethnic difference.

There was no difference in the plasma adiponectin level according to *ACDC* genotypes in this study. This suggests that the association between SNP45 and carotid atherosclerosis may not be directly mediated by the alteration in plasma adiponectin levels. SNP45 and SNP276 have been

Table 4
Adiponectin genotype distribution and haplotype frequency and the association with the risk of CAP

	CAP			Total	Dominant			Recessive			Multiplicative		
	Yes	No	*P*[a]		OR[b]	(95% CI)	*P*[b]	OR[b]	(95% CI)	*P*[b]	OR[b]	(95% CI)	*P*[b]
Genotype	397	311		708									
SNP45													
TT	185 (46.6%)	161 (51.8%)		346 (48.9%)									
TG	174 (43.8%)	132 (42.4%)		306 (43.2%)									
GG	38 (9.6%)	18 (5.8%)	.12[a]	56 (7.9%)	1.350	(0.953-1.912)	.27	2.468	(1.195-5.098)	.045	1.410	(1.064-1.870)	.05
SNP276													
GG	209 (52.6%)	142 (45.7%)		351 (49.6%)									
GT	158 (39.8%)	139 (44.7%)		297 (41.9%)									
TT	30 (7.6%)	30 (9.6%)	.16[a]	60 (8.5%)	0.737	(0.520-1.044)	.27	0.657	(0.348-1.241)	.60	0.765	(0.581-1.007)	.18

[a] *P* values for differences in genotype distribution between patients with and without CAP were calculated by the χ^2 test.

[b] Odds ratio (95% CI) and corresponding *P* value for logistic regression of risk of carotid plaque in 3 different inheritance models, adjusting for age, sex, BMI, diabetes duration, HDL cholesterol, fasting plasma glucose, 2-hour PPG, current smoking status, and systolic blood pressure, are shown. The common alleles were used as reference genotypes to heterozygotes and homozygotes of the minor allele. *P* values were adjusted for multiple comparison using the Bonferroni method.

associated with serum adiponectin levels in some studies [12], but others have shown no association with serum adiponectin levels [37,38]. Consistent with our result, Bacci et al [15] also reported that there was no association between plasma adiponectin level and *ACDC* genotype, although coronary artery disease was associated with *ACDC* genotype. In addition, Lee et al [38] also reported no difference in plasma adiponectin levels according to SNP45 and SNP276 in Korean patients with type 2 diabetes mellitus, which is in accordance with our results. There are several possible explanations for the absence of any difference in adiponectin levels despite the difference in CVDs or carotid atherosclerosis according to genotype. First, the current plasma adiponectin levels measured in this study may not reflect levels when atherosclerosis started to develop. It is also unclear whether serum concentrations reflect adiponectin levels in the vascular wall, which is believed to be more important in terms of antiatherogenic effects for adiponectin [39,40]. Moreover, we previously have shown that the response to rosiglitazone differs according to adiponectin genotypes [21]. The different response to different drugs or stimuli according to adiponectin genotype and/or haplotype may be another possible mechanism influencing plaque formation. These matters should be investigated further to understand the mechanism by which adiponectin polymorphisms affect carotid atherosclerosis.

In conclusion, this study is the first to report an association between the *ACDC* polymorphism SNP45 T>G and CAP. Our finding may provide new knowledge in the understanding of the genetics of ultrasound-defined carotid atherosclerosis. It may also aid in identifying patients with type 2 diabetes mellitus with an increased risk for macrovascular complications and those who are in need of intensive treatment, and aid in developing effective treatment strategies.

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