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The SNP276G>T polymorphism in the adiponectin (*ACDC*) gene is more strongly associated with insulin resistance and cardiovascular disease risk than SNP45T>G in nonobese/nondiabetic Korean men independent of abdominal adiposity and circulating plasma adiponectin

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Abstract

This study aimed to determine whether polymorphisms of the adiponectin (*ACDC*) gene independently contribute to insulin resistance (IR) and other cardiovascular disease (CVD) risk factors in nonobese, nondiabetic Korean men after adjusting for major environmental factors that influence IR. Among the 7 *ACDC* single-nucleotide polymorphisms (SNPs;C-11377G, T45G, G276T, H241P, Y111H, G90S, and R221S) prescreened in 48 subjects, we genotyped 333 subjects for SNP45 and SNP276, both of which showed an allele frequency of more than 2%. In Pearson correlation and multiple stepwise regression analyses, we found that waist circumference was the most important influencing factor (β = .369, P < .001) in homeostasis model assessment (HOMA)–IR, whereas plasma adiponectin was the second most important (β = −.217, P = .023). At position 276, T/T subjects showed significantly lower glucose concentrations (P = .043) and higher low-density lipoprotein particle sizes (P = .033) than the G/G and G/T subjects. The subjects also had lower serum triglycerides and HOMA-IR; however, these results were not statistically significant. After adjusting for waist circumference and plasma adiponectin, T/T subjects showed a significantly lower HOMA-IR than G/G or G/T subjects (P = .048). On the other hand, at position 45, only glucose concentrations were significantly lower in G carriers (P = .005). In the SNP45-SNP276 haplotype test, TT/TT subjects (having T/T at both SNP45 and SNP276) showed significantly lower IR before and after adjusting for waist circumference and adiponectin levels than did other carriers. In conclusion, we suggest that SNP276G>T, rather than SNP45T>G, is more strongly associated (both directly and indirectly) than with several components of metabolic syndrome and CVD risk, including IR, triglyceride concentration, and low-density lipoprotein particle size, in nonobese, nondiabetic men.

1. Introduction

Insulin resistance (IR) is a major factor responsible for the development of type 2 diabetes mellitus (T2DM) and the increase of obesity-associated cardiovascular disease (CVD) risk, particularly abdominal obesity [1-4]. It occurs not only in patients with diabetes, but also in prediabetic patients and those in normoglycemic states [5]. Recently, many studies have reported that circulating adiponectins (proteins produced in adipose tissue) known as apM1, GPB28, Acrp30, and AdipoQ are negatively associated with components of metabolic syndrome [6-8] and are lower in obese subjects and patients with T2DM or coronary artery disease (CAD) than in healthy, normoglycemic subjects [9,10]. On the other hand, Weyer et al [11]

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reported a stronger relationship between adiponectins and whole-body insulin sensitivity than between adiponectins and body fat distribution. Circulating adiponectin levels are also inversely correlated with cytokines, such as C-reactive protein (CRP), interleukin-6, and tumor necrosis factor α, which are closely related to CVD risk [12-17]. Thus, adiponectins have been suggested to have multiple protective roles as antidiabetic, anti-atherosclerotic, and anti-inflammatory factors [6].

According to recent gene association studies, the *ACDC* (adipocyte-, C1Q-, and collagen domain-containing) gene regulates the production of adiponectin in adipose tissue and modulates circulating plasma adiponectin. A decrease or deficiency in adiponectin probably causes IR, thereby leading to the development of T2DM and CVD [18,19]. This gene consists of 3 exons and 2 introns localized on chromosome 3q27, where a susceptibility locus for T2DM and adiposity measures have been mapped [20,21]. Two common *ACDC* single-nucleotide polymorphisms (SNPs), 45T>G in exon 2 and 276G>T in intron 2, are associated with T2DM in Japanese patients [18]. The 276G>T polymorphism, observed in diabetic whites, was a determinant of CAD risk [22]. In nondiabetic whites, the SNP 45T>G has been significantly associated with obesity and IR syndrome as an independent SNP [23] or as a haplotype when combined with 276G>T [19]. However, an association between these 2 SNPs and IR has not been observed in Swedish [24] or French populations [25]. Therefore, it has been suggested that susceptibility to IR or adiposity in those with this locus may be heterogeneous among ethnic groups [22].

The aim of this study was to identify whether the 2 aforementioned polymorphisms in the *ACDC* gene, 45T>G and 276G>T, independently contribute to circulating adiponectin levels, IR, or other CVD risk factors in nonobese, nondiabetic Korean men after adjustments for major environmental factors that contribute to IR.

2. Subjects and methods

2.1. Study subjects

A total of 333 volunteer study subjects were recruited after responding to advertisements for a nutritional genomic study conducted by the Clinical Nutrition Research Laboratory at Yonsei University and the Cardiovascular Genome Center at Yonsei University Hospital. All patients were nonobese (body mass index [BMI] ≤ 30 kg/m^2) and nondiabetic men based on the American Diabetes Association criteria [26] in which diabetes is defined as a fasting plasma glucose level of 126 mg/dL or more (≥7 mmol/L) or treated with antidiabetic agents. None of the subjects were taking medication or had been diagnosed with CVD, DM, or cancer. Written informed consent was obtained from all subjects. The study protocol was approved by the Institutional Review Board of Yonsei University.

2.2. Anthropometric and blood pressure measurements

Body weight and height were measured unclothed and without shoes in the morning. BMI was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Body fat percentages were measured with a TBF-105 body fat analyzer (Tanita, Tokyo, Japan). Waist and hip circumferences were measured with paper tape in the standing position after normal expiration. Waist circumferences were measured horizontally at the umbilicus, whereas hip circumferences were measured horizontally around the largest part of the hips and buttocks. These parameters were measured by well-trained dietitians who have considerable experience in clinical research. Before the start of this study, we evaluated errors among the measurers and found that it was within 0.05%. Blood pressure was read from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 minutes of rest. The average of 3 measurements was recorded for each subject.

2.3. Blood collection

Venous blood specimens were collected in EDTA-treated and plain tubes after a 12-hour fast. The tubes were immediately covered with aluminum foil and placed on ice until they arrived at the laboratory room (within 1-3 hours) and were stored at −70°C until analysis.

2.4. Genotyping

Genomic DNA was extracted from 5-mL whole blood using a commercially available DNA isolation kit (WIZARD Genomic DNA purification kit, Promega, Madison, WI) according to the manufacturer's protocol. We first prescreened 7 sites from previously reported *ACDC* SNPs (G-11391A at the proximal promoter; T45G at exon 2; G276T at intron 2; and H241P, Y111H, G90S, and R221S at exon 3) in 48 subjects to identify the allele frequency of each SNP. Each genotyping reaction was performed with SNP-IT assays using single primer extension technology (SNPstream 25K System, Orchid Biosystems, Princeton, NJ). The DNA fragments were visualized by UV illumination using an Image Analyzer (AlphaImager 1220, Alpha Innotech, San Leandro, CA). pUC19 DNA/*Msp*I (*Hpa*II) Marker (MBI Fermentas, Vilnius, Lithuania) served as a control standard.

2.5. Serum lipid profile

Fasting serum concentrations of total cholesterol and triglycerides (TGs) were measured using commercially available kits and a Hitachi 7150 Autoanalyzer (Hitachi, Tokyo, Japan). After precipitation of serum chylomicrons, low-density lipoproteins (LDL), and very low-density lipoproteins using dextran sulfate–magnesium, the high-density lipoprotein cholesterol (HDL-C) left in the supernatant was measured using an enzymatic method. LDL-C was estimated indirectly using the Friedewald formula

Table 1
General characteristics of 333 nonobese, nondiabetic Korean men

Age (y)	40.1 ± 0.77
Body weight (kg)	71.3 ± 0.59
Height(cm)	171.1 ± 0.34
BMI (kg/m^2)	24.3 ± 0.17
Waist (cm)	87.3 ± 0.47
WHR	0.88 ± 0.00
Percentage of body fat	23.0 ± 0.31
Systolic blood pressure (mm Hg)	125.7 ± 0.84
Diastolic blood pressure (mm Hg)	77.5 ± 0.64
Cigarette smoking (per day)	16.0 ± 0.56
TGs (mg/dL)	147.9 ± 4.64
Total cholesterol (mg/dL)	199.3± 2.03
HDL-C (mg/dL)	46.1 ± 0.59
LDL-C (mg/dL)	123.6 ± 1.87
Glucose (mg/dL)	87.3 ± 0.68
Insulin (μIU/mL)	8.45 ± 0.26
HOMA-IR	1.83 ± 0.06

Data are presented as mean ± SE. N = 333.

for subjects with a serum TG concentration of less than 400 mg/dL and was directly measured for subjects with a serum TG concentration of 400 mg/dL or more.

2.6. Glucose, insulin, free fatty acids, and HOMA-IR

Fasting glucose was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by radioimmunoassays with commercial kits from Immuno Nucleo (Stillwater, MN). Free fatty acids were analyzed with a Hitachi 7150 autoanalyzer (Hitachi). Insulin resistance was calculated with the homeostasis model assessment (HOMA) using the following equation: HOMA-IR = [fasting insulin (μIU/mL) × fasting glucose (mmol/L)]/22.5 [27].

2.7. LDL particle size and plasma levels of adiponectin, CRP, and malondialdehyde

An LDL particle size distribution (d = 1.019-1.063 g/mL) was performed by sequential floatation ultracentrifugation and examination using a pore gradient lipoprotein system (CBS Scientific, Del Mar, CA) and commercially available nondenaturing polyacrylamide slab gels containing a linear gradient of 2% to 16% acrylamide (Alamo Gels, San Antonio, TX). Standards of latex beads (34 nm), thyroglobulin (17 nm), apoferritin (12.2 nm), and catalase (10.4 nm) were used to estimate the relative migration (Rf) rates of each band. The gels were scanned with a GS-800 calibrated imaging densitometer (Bio-Rad Laboratories, Graz, Austria). LDL particle size was calculated by referring to the Rf values of the standards. Plasma adiponectin concentrations were measured by an enzyme immunoassay (Human Adiponectin enzyme-linked immunoassay kit, B-Bridge International, Sunnyvale, CA). The resultant color reaction was read using a Victor^2 (Perkin Elmer Life Sciences, Turka, Finland) at 450 nm. Plasma CRP was measured with an Express Plus autoanalyzer (Chiron Diagnostics, Walpole, MA) using a commercially available high-sensitivity CRP-Latex (II) X2 kit (Seiken Laboratories, Tokyo, Japan) [28]. Plasma malondialdehyde was measured according to the fluorometric method described by Miller et al [29].

2.8. Statistical analysis

We used SPSS version 12.0 for Windows (SPSS Institute, Chicago, IL) for all of our statistical analyses. Pearson correlation tests and multiple stepwise regression analyses were performed to find the major factors influencing IR. Among the prescreened *ACDC* SNPs, we excluded SNPs with an allele frequency lower than 2%. All variables in each SNP group were tested using analysis of variance (ANOVA) followed by the Bonferroni method. To find the genetic effect on HOMA-IR and CVD risk, we used a general linear model, adjusting for the major factors as mentioned above. Before statistical tests, each variable was examined to ensure normal distribution; significantly skewed variables were log-transformed. For descriptive purposes, mean values are presented on untransformed and unadjusted variables. Results are expressed as mean ± SE. A 2-tailed P value of less than .05 was considered statistically significant.

3. Results

3.1. General characteristics of study subjects

Table 1 presents the general characteristics of the 333 nonobese, nondiabetic men. The mean age was 40.1 ± 0.77 years, and the mean BMI was 24.3 ± 0.17 kg/m^2. Mean values of waist circumference, waist-to-hip ratio (WHR), percentage of body fat, blood pressure, and biochemical parameters (TG, total cholesterol, HDL-C, LDL-C, glucose, and insulin) were all within reference ranges.

3.2. Pearson correlation of HOMA-IR and plasma adiponectin with anthropometric parameters and metabolic variables

HOMA-IR was positively correlated with several basic anthropometric parameters: waist circumference (r = 0.483,

Table 2
Pearson correlation of IR and plasma adiponectin with anthropometric and metabolic parameters

	HOMA-IR[a]		Adiponectin[a]	
	R	P	R	P
Age	−0.042	.469	0.032	.690
BMI	0.475	<.001	−0.292	<.001
Waist circumference	0.483	<.001	−0.316	<.001
Percentage of body fat	0.445	<.001	−0.340	<.001
Cigarette smoking (per day)	0.192	.007	−0.040	.686
Systolic blood pressure	0.217	<.001	−0.188	.017
Diastolic blood pressure	0.227	<.001	−0.225	.004
TG[a]	0.277	<.001	−0.329	<.001
HDL-C	−0.340	<.001	0.238	.002
LDL particle size	−0.162	.024	0.311	.002
Fasting glucose	0.423	<.001	−0.159	.044
CRP[a]	0.244	<.001	−0.164	.059
Adiponectin[a]	−0.284	<.001	–	–

[a] Log-transformed.

Table 3
Stepwise multiple regression analyses to identify factors influencing IR in nonobese, nondiabetic Korean men

Dependent variable	Model	Independent variable	Unstandardized		Adjusted	P	R	P
			β	Constant	β			
HOMA -IR[a]	1 Step	Waist circumference	.031	−2.302	.464	<.001	0.464	<.001
	2 Steps	Waist circumference	.006	−1.657	.396	<.001	0.508	<.001
		Adiponectin[a]	−.215		−.217	.023		

The independent variables were age, BMI, waist circumference, percentage of body fat, systolic and diastolic blood pressure, adiponectin, and cigarette smoking.
[a] Log-transformed.

$P < .001$), BMI ($r = 0.475$, $P < .001$), percentage of body fat ($r = 0.445$, $P < .001$), systolic blood pressure ($r = 0.217$, $P < .001$), diastolic blood pressure ($r = 0.227$, $P < .001$), and cigarette smoking ($r = 0.192$, $P < .001$) (Table 2). Among biochemical parameters, HOMA-IR also correlated positively with fasting glucose ($r = 0.423$, $P < .001$), serum TGs ($r = 0.277$, $P < .001$), and CRP ($r = 0.244$, $P < .001$). However, it correlated negatively with serum HDL-C ($r = -0.340$, $P < .001$), plasma adiponectin ($r = -0.284$, $P < .001$), and LDL particle size ($r = -0.162$, $P = .024$). Correlation patterns of plasma adiponectin with the above parameters were contrary to those of HOMA-IR (Table 2).

3.3. Multiple stepwise regression analysis to identify influencing factors on HOMA-IR

Table 3 shows the major influencing factors for HOMA-IR from the anthropometric parameters and plasma adiponectin shown in Table 2. In the multiple stepwise regression model, we found that waist circumference was the most important influencing factor for HOMA-IR (adjusted $\beta = .396$, $P < .001$), whereas plasma adiponectin was the second (adjusted $\beta = -.217$, $P < .023$) (Table 3). These 2 parameters were used for adjustment in the comparison of mean values according to genotype.

3.4. Detection of SNPs in the ACDC gene

Among the prescreened *ACDC* SNPs, we found 5 rare, nonsynonymous mutations (G-11391A, H241P, Y111H, G90S, R221S; allele frequency <2%). The allele frequencies of these SNPs were as follows: G-11391A (G/A = 1:0), H241P (A/C = 1:0), Y111H (T/C = 1:0), G90S (G/A = 1:0), R221S (C/A = 0.98:0.02). Therefore, we included the SNPs T45G and G276T in further analyses.

3.5. Frequency of the 45T>G and 276G>T ACDC polymorphisms

Among the 333 study subjects, 156 subjects were homozygous at position 45 for the T allele (T/T), whereas 146 were heterozygous for the G allele (T/G) and 31 were homozygous for G allele (G/G) (T frequency = 0.69). At position 276, 170 subjects were homozygous for the G allele (G/G), 141 were heterozygous for the T allele (G/T), and 22 were homozygous for the T allele (T/T) (G frequency = 0.72). These genotype distributions were in Hardy-Weinberg equilibrium ($P = .703$ at 45T>G, $P = .306$ at 276G>T). SNP45 and SNP276 were found to be highly linked by the linkage disequilibrium test ($d' = -1.000$, $P < .001$). There were 58 homozygous carriers of the TG haplotype (TG/TG; individuals who were T/T at SNP45 and G/G at SNP276), 22 homozygous carriers of the TT

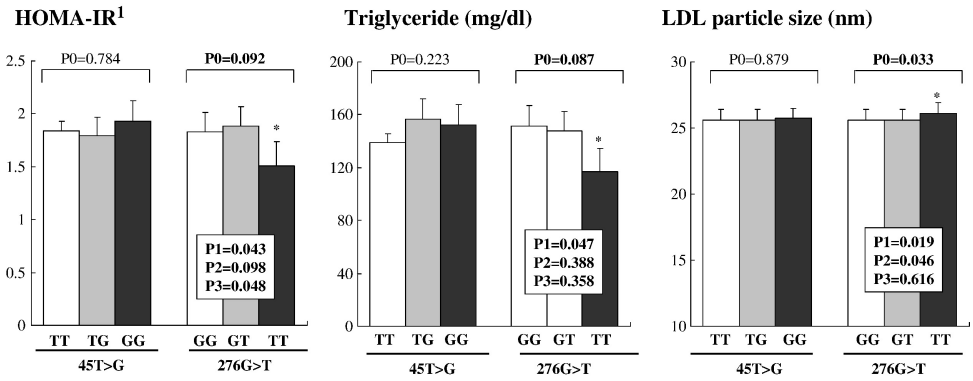

Fig. 1. HOMA-IR, serum TG concentration, and LDL particle size according to genotype before and after adjustment. Tests were performed by ANOVA with the Bonferroni method and analysis of covariance with a general linear model adjusting for covariates. *Value is significantly different from the others in the same SNP when $P < .05$ in any test; P_0, before adjustment; P_1, adjusted for waist circumference; P_2, adjusted for adiponectin; P_3, adjusted for waist circumference and adiponectin.

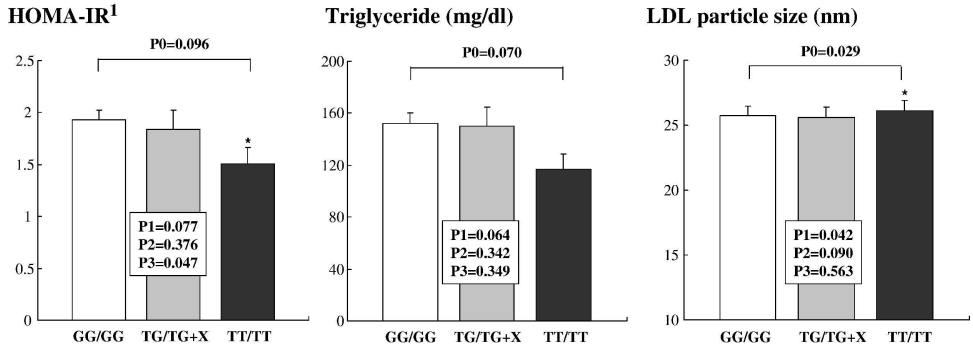

Fig. 2. HOMA-IR, serum TG concentration, and LDL particle size according to haplotype before and after adjustment. Tests were performed by ANOVA with the Bonferroni method and analysis of covariance with a general linear model adjusting for covariates. *Value is significantly different from the others when $P < .05$ in any test; P_0, before adjustment; P_1, adjusted for waist circumference; P_2, adjusted for adiponectin; P_3, adjusted for waist circumference and adiponectin.

haplotype (TT/TT), 31 homozygous carriers of the GG haplotype (GG/GG), and 222 heterozygous carriers of the TG haplotype (TG/X).

3.6. Anthropometric parameters, serum lipid profiles, LDL particle size, cytokines, lipid peroxide, and fasting glucose levels according to genotype

There were no genotype-related differences in age, weight, BMI percentage of body fat, waist circumference, WHR, blood pressure, serum concentrations of total, HDL-C, or LDL-C, or plasma concentrations of adiponectin, CRP, and MDA at position 45 or 276 (data not shown). Compared with G carriers of both SNP45 and SNP276, T/T subjects of both SNPs showed significantly lower concentrations of fasting glucose (T/T, 84.9 ± 0.90; T/G, 89.4 ± 1.12; G/G, 89.2 ± 2.03 at SNP45, $P = .005$; G/G, 86.9 ± 0.91; G/T, 88.6 ± 1.12; T/T, 81.7 ± 1.99 at SNP276, $P = .0043$).

3.7. HOMA-IR, serum TG concentration, and LDL particle size according to genotype before and after adjustments for waist circumference and plasma adiponectin

Fig. 1 shows the effects of genotype on HOMA-IR, serum TG concentration, and LDL particle size before and after adjustment. HOMA-IR was significantly lower in subjects with T/T at SNP276 after adjusting for waist circumference ($P_1 = .043$) and for both waist circumference and plasma adiponectin ($P_3 = .048$). Serum TG concentrations in this group were also significantly lower ($P_1 = .047$) after adjusting for waist circumference; however, the significance disappeared after adjusting for plasma adiponectin ($P_2 = .338$) and for both waist circumference and adiponectin ($P_3 = .358$). LDL particle size was significantly higher in subjects with T/T at SNP276 before and after adjusting for waist circumference ($P_1 = .019$) and for plasma adiponectin ($P_2 = .046$); however, the significance disappeared after adjusting for both waist circumference and plasma adiponectin ($P_3 = .616$). On the other hand, there were no differences in these variables based on the genotype at position 45.

3.8. Effects of the SNP45 and SNP276 haplotype on HOMA-IR, serum TG concentration, and LDL particle size before and after adjustments

As SNP45 and SNP276 were in linkage disequilibrium ($d' = -1.000$, $P < .001$), study subjects were categorized into 3 haplotype groups: (1) the TT/TT group (homozygous TT haplotype), (2) the GG/GG group (homozygous GG haplotype), and (3) the TG/TG + X group (both homozygous and heterozygous TG haplotypes). There were no significant differences in age, body weight, BMI, waist circumference, or percentage of body fat among these 3 groups (data not shown). Compared with the GG/GG and TG/TG + X groups, the TT/TT group had a lower HOMA-IR after adjustments for both waist circumference and plasma adiponectin ($P_3 = .047$; Fig. 2). The TT/TT group also showed a significantly higher LDL particle size before and after adjusting for waist circumference ($P_0 = .029$, $P_1 = .042$). However, these significant differences disappeared after adjusting for plasma adiponectin and for both waist circumference and adiponectin levels. The TT/TT group seemed to have lower concentrations of serum TGs before and after adjusting for waist circumference; however, it was not statistically significant ($P = .070$ and .064, respectively).

4. Discussion

In the present study, we confirmed that *ACDC* SNP 276G>T, rather than SNP 45T>G, is more strongly associated (both directly and indirectly) with several components of metabolic syndrome and CVD, including IR, serum TG concentration, and LDL particle size, in nonobese, nondiabetic Korean men.

This study showed that G carriers at SNP276 had higher IR than T/T subjects after adjustments for waist circumference and plasma adiponectin, both of which were closely correlated with IR. This pattern was also observed in the SNP45-SNP276 haplotype test; compared

with the GG/GG and TG/TG + X subjects, homozygous TT/TT subjects, who have T/T at both SNP45 and SNP276, showed a significantly lower HOMA-IR after adjustment. Thus, the G allele at position 276 might be more susceptible to T2DM and CVD than T/T homozygotes in a nonobese, nondiabetic state. However, we could not find any significant relationship between SNP45 and IR, although G carriers at position 45 had significantly higher fasting glucose concentrations than T/T subjects. These results are consistent with the previous reports by Menzaghi et al [19], Populaire et al [30], and Bacci et al [31]. Although SNP276 is located in an intronic region with no apparent biological function, this intronic SNP may affect the expression level of the gene via an unknown mechanism, similar to the actions of intronic SNPs in Calpin10 and COL1A1 [32,33]. However, we did not directly measure the expression levels of adiponectin in our subjects. Another possible explanation is that this SNP is in linkage disequilibrium with other still undiscovered SNP(s) in the *ACDC* gene or other genes that have biological effects on IR. On the other hand, SNP45, located in exon 2 of the *ACDC* gene and coding for a silent mutation, Gly15Gly (GGT to GGG) [34], might not strongly predispose nonobese, nondiabetic individuals to T2DM or CVD compared with SNP276.

However, not all studies are in agreement with our results. Hara et al [18] demonstrated that nonobese, nondiabetic, elderly (>60 years) Japanese subjects with G alleles at both SNP45 and SNP276 had a significantly higher risk of T2DM. A German population study showed that the G allele at locus 45 was associated with obesity and the risk of DM [23]. According to Yang et al [35], the G allele of SNP45 may have higher transcriptional activity than the T allele because of altered mRNA splicing or stability that possibly inactivates the *ACDC* gene. Zacharova et al [36] suggested that the G allele of SNP45 is a predictor for the conversion to T2DM and that the combined effect of the SNP45 G allele and SNP276 T allele on T2DM development was stronger than that of each SNP alone. He also reported that women carrying the risk genotype combination had a higher risk of conversion to DM than men [36]. On the other hand, a French population study reported that SNP-11391G>A and SNP-11377G>C were more strongly related to DM and plasma adiponectin levels than SNP45 and SNP276 [25]. Considering these results, the susceptibility of SNPs in the *ACDC* gene for IR, T2DM, or CVD may differ not only from population to population, but also from environmental factors, such as sex, age, degree of obesity, diabetic or prediabetic state, and so on. Our study subjects were all middle-aged, nonobese, nondiabetic men. Therefore, more prospective or longitudinal intervention studies are necessary to identify the independent effects of each *ACDC* genotype.

As reported in other studies [12,15-17,37], this study also showed that circulating adiponectin levels correlated positively with HDL-C and LDL particle size and negatively with HOMA-IR, waist circumference, BMI, blood pressure, and fasting concentrations of glucose, TG, and CRP. According to Cnop et al [38], insulin sensitivity is related to intra-abdominal fat and adiponectin, whereas adiponectin is associated with body fat distribution (particularly intra-abdominal fat accumulation), sex, and age. Therefore, he suggested that both intra-abdominal fat and adiponectin contribute independently to TGs, HDL-C, and LDL particle size, all of which were closely related to IR. According to in vitro experiments, adiponectin accumulates in injured vessel walls [13] and inhibits tumor necrosis factor α–induced monocyte adhesion and expression of adhesion molecules in endothelial cells [14], thereby protecting against obesity, IR, T2DM, and atherosclerosis. Yamauchi et al [39] showed in animal experiments that long-term treatment (12 days) with adiponectin improved insulin sensitivity, reduced the storage of TGs in the liver and muscle, and increased protein expression in muscle through the combustion and dissipation of fatty acids.

A number of studies have suggested that *ACDC* gene polymorphisms affect IR and T2DM development by regulating adiponectin levels. However, we did not observe an association between adiponectin concentration and *ACDC* genotype at position 276 or 45. Takahashi et al [34] showed that the mean level of plasma adiponectin tended to be low in Japanese men and women carrying the GG genotype at position 45. Fumeron et al [40] reported a trend toward higher levels of circulating adiponectin in women carrying the 45G allele. Menzaghi et al [19] showed that TG/TG homozygotes in the SNP45T>G/276G>T haplotype had significantly lower plasma adiponectin levels and higher IR independent of sex, age, and body weight. On the other hand, reports by Bacci et al [31] and Hara et al [18] are more similar to our study results. Bacci et al [31] reported that patients with T2DM who were T/T homozygotes at SNP276 were at lower risk of CAD than carriers of the G allele; however, there were no significant differences in adiponectin concentration between genotype groups. According to the report by Hara et al [18], adiponectin concentrations did not differ significantly in the SNP45 or SNP276 genotypes, although these SNPs are related to IR. However, the association between the SNP276 genotype and adiponectin levels was shown only in relatively obese individuals (BMI > 26.7). Considering that none of our study subjects were obese, it is possible that we could not find any significant differences in adiponectin concentration between SNP276 genotypes. However, we did not perform subset analysis according to BMI because of the relatively small number of subjects with the SNP276 T/T genotype.

Although we could not exactly explain why the relationship between SNP276 and IR was observed among subjects regardless of adiponectin concentration, we assumed that these divergences include population differences in sex, BMI, and age. Our study subjects were all

middle-aged, nonobese, nondiabetic men. On the other hand, other studies included men and women [34,36,40]; those with normoglycemia, impaired glucose tolerance, and T2DM [19,36,40]; overweight and obese people [34,36]; and/or subjects from a variety of age groups [19,36]. It is particularly interesting that Japanese and Koreans showed a somewhat different response to the SNP45 or SNP276 genotype, although the 2 populations have been geographically close for a long period. There have been reports of 2 populations having relatively similar genetic background to Western or other continental people; however, genetic patterns are not likely to be 100% equal to each other [41]. Regarding this aspect, we consider the possibility that differences in SNP45 and SNP276 of the *ACDC* gene exist between 2 populations. Another reasonable explanation is that plasma adiponectin may not necessarily reflect adiponectin concentrations in the subendothelial space, where the targets for the anti-atherogenic effect of these cytokines are located [42-44]. In this study, we measured adiponectin only from the plasma level; thus, we might not have been able to find a significant association between SNP276 and adiponectin levels. According to Fruebis et al [45], adiponectin phenotypes can affect adiponectin activity. There are 2 types of adiponectin: one is full length (ACRP30) and the other is a globular head protein (gACRP30). Among the 2 phenotypes, gACRP30 is more active in regulating plasma concentrations of glucose, free fatty acids, and TGs and ameliorating IR [39,45]. Therefore, we need further studies of not only adiponectin concentrations at the subendothelial level, but also for specific adiponectin phenotypes according to genotype.

As stated above, fasting TG and LDL particle size are associated with IR [46,47] and are regulated by adiponectin levels [37,48]. It may be of interest that subjects with the T/T genotype at position 276 having tendencies toward lower TG concentrations and significantly higher LDL particle size than those with the G/G or G/T genotypes lost such tendencies after adjustments for plasma adiponectin or for both waist circumference and adiponectin levels. This pattern was also shown in the SNP45-SNP276 haplotype test. From these results, we assumed that serum TG concentration and LDL particle size are not directly regulated by the SNP276 genotype itself, but are more strongly influenced by the degree of IR or the possibility of T2DM, as shown in the SNP276 genotype group.

In summary, we suggest that the *ACDC* SNP 276G>T is more strongly associated with IR and CVD risk than SNP 45T>G in nonobese, nondiabetic Korean men, even after adjusting for circulating plasma adiponectin and abdominal obesity. Longitudinal prospective and intervention studies, along with functional studies, are necessary to identify the exact role of *ACDC* gene polymorphisms in IR syndrome, T2DM, and CVD risk in nonobese, nondiabetic Korean people.

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