Original Article

Adiponectin Genetic Variability, Plasma Adiponectin, and Cardiovascular Risk in Patients With Type 2 Diabetes

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Adiponectin is an adipocyte-derived hormone that has shown anti-inflammatory and antiatherogenic effects. We assessed the associations of variants in the adiponectin gene (*ADIPOQ*) with circulating adiponectin levels and cardiovascular risk among women with type 2 diabetes. Of 989 diabetic women from the Nurses' Health Study, 285 developed cardiovascular disease (CVD) during follow-up through 2000. We genotyped five *ADIPOQ* polymorphisms in the CVD case and control subjects. A promoter polymorphism −11365C→G was significantly associated with lower plasma adiponectin levels ($P = 0.004$). The homozygotes of allele −4034C were significantly associated with ~60% increased cardiovascular risk (odds ratio 1.62 [95% CI 1.07–2.45]). Adjustment for age, BMI, and other covariates did not appreciably change the associations. In addition, a common haplotype possessing allele +276T (CAATT) was associated with a significantly lower CVD risk than the most common haplotype (CAATG) (0.70 [0.50–0.98]). In our meta-analysis of 827 CVD case and 1,887 CVD-free control subjects, polymorphism +276G→T was significantly associated with ~45% (20–62%) decreased CVD risk under a recessive inheritance mode in diabetic patients. In conclusion, *ADIPOQ* promoter polymorphism −11365C→G was associated with plasma adiponectin levels, whereas polymorphisms −4034A→C and +276G→T were associated with CVD risk in diabetic patients. *Diabetes* 55:1512–1516, 2006

Adiponectin is the most abundant adipocyte-secreted hormone in blood (1). Adiponectin improves insulin action and the metabolism of glucose and lipids (2,3). The adiponectin levels are decreased in patients with obesity and type 2 diabetes (4,5). In addition, adiponectin may regulate inflammatory response (6) and has an antiatherogenic effect (7,8). A strong linkage has been reported between the chromosomal region encompassing adiponectin gene (*ADIPOQ*) and cardiovascular risk factors (9,10).

Recently, Lacquemant et al. (11) reported that polymorphism +45T→G in the *ADIPOQ* gene was associated with an increased risk of coronary artery disease among patients with type 2 diabetes. In another study (12), polymorphism +276G→T was associated with a decreased coronary artery disease risk. However, these studies have been limited by the small sample size and case-control retrospective design and need to be replicated in large prospective studies.

In an earlier effort to replicate these findings in a prospective cohort of diabetic men, we found that *ADIPOQ* polymorphism +276G→T was associated with higher plasma adiponectin levels and a reduced cardiovascular disease (CVD) risk (13). Women and men have different circulating levels of adiponectin (14). In this study, we sought to investigate the associations of *ADIPOQ* genetic variability with blood adiponectin levels and CVD risk in women with type 2 diabetes. We genotyped five common polymorphisms in the *ADIPOQ* gene (−11365C→G, −4034A→C, −3964A→G, +45T→G, and +276G→T) among diabetic women from the Nurses' Health Study.

RESEARCH DESIGN AND METHODS

The Nurses' Health Study began in 1976 with the recruitment of 121,700 female registered nurses between the ages of 30 and 55 years. The information of lifestyle factors and disease diagnosis is updated by validated questionnaires every 2 years. A total of 32,826 women provided blood samples during 1989 and 1990. At blood collection, most participants (85%) had diagnosed diabetes, and the rest were diagnosed before 1994. We used National Diabetes Data Group criteria to define diabetes because our subjects were diagnosed before the release of the American Diabetes Association criteria in 1997 (15). The validity of this method has been confirmed (16). A case of diabetes was considered if at least one of the following was reported on the supplementary questionnaire: *1*) classic symptoms plus elevated fasting plasma glucose ≥7.8 mmol/l, random plasma glucose ≥11.1 mmol/l, and/or plasma glucose ≥11.1 mmol/l after ≥2 h during an oral glucose tolerance test; *2*) no symptoms but at least two elevated plasma glucose concentrations (by the above criteria) on different occasions; or *3*) treatment with oral hypoglycemic agents or insulin. **Ascertainment of CVDs.** Subjects were classified as having CVD if they had confirmed fatal coronary heart disease, nonfatal myocardial infarction, and stroke or had experienced coronary artery bypass grafting or percutaneous

TABLE 1
Baseline characteristics of diabetic women in CVD case and control subjects

	CVD case subjects	Control subjects	P
n	285	704	—
Age (years)	47	44	<0.01
BMI (kg/m^2)	29.1	27.7	<0.01
Current smoker (%)	31.2	25.7	0.20
Alcohol intake (g/day)	7.2	6.7	0.58
Physical activity (MET hours/week)	10.1	12.2	0.07
Family history of coronary heart disease (%)	25.8	21.7	0.17
History of hypertension (%)	37.5	25.1	<0.01
History of high cholesterol (%)	10.9	6.8	0.03
Postmenopausal (%)	87.7	77.7	<0.01

transluminal coronary angioplasty. Nonfatal myocardial infarction was confirmed by reviewing medical records using the criteria of the World Health Organization of symptoms plus either typical electrocardiographic changes or elevated levels of cardiac enzymes. Stroke was confirmed by reviewing medical records using the criteria of the National Survey of Stroke. Physicians who reviewed the records had no knowledge of the self-reported risk factors. Cardiovascular deaths were confirmed by review of medical records or autopsy reports with the permission of the next of kin. Sudden deaths were included in the fatal coronary heart disease category. We included the CVD case subjects who were diagnosed as having CVD after the onset of diabetes and through 2000. We excluded those missing all ADIPOQ genotypes. Finally, a total of 989 women (285 CVD case and 704 control subjects) were included in this study.

Assessment of biochemical markers and covariates. Blood was collected during 1989 and 1990. Plasma adiponectin concentration was measured by competitive radioimunoassay (Linco Research, St. Charles, MO) with a coefficient of variation of 3.4% (17); BMI was calculated as weight in kilograms divided by the square of height in meters. Physical activity was expressed as MET hours based on self-reported types and durations of activities over the previous year (18).

Genotype determination. DNA was extracted from the buffy coat fraction of centrifuged blood with the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Four single nucleotide polymorphisms (SNPs), −11365C→G (rs266729), −4034A→C (rs822395), +45T→G (rs2241766), and +276G→T (rs1501299), were chosen for their ability to tag all common haplotypes at the adiponectin locus (19). Another SNP, −3964A→G (rs822396), was selected because of its lack of strong linkage disequilibrium (LD) with the four haplotype-tag SNPs (20). The polymorphisms were genotyped with Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Replicate quality control samples were included and genotyped with 100% concordance.

Statistical analyses. A χ^2 test was used to assess whether the genotypes were in Hardy-Weinberg equilibrium and to determine differences in genotype frequencies between CVD case and control subjects. Unconditional logistic regression was used to calculate odds ratios (ORs), adjusting for age, BMI, smoking, alcohol consumption, physical activity, HbA$_{1c}$, history of hypertension or hypercholesterolemia, duration of diabetes, and postmenopausal hormone use. General linear models were used to compare geometric mean values of quantitative traits across groups. Plasma adiponectin was not

normally distributed and was logarithmically transformed to improve the normality. The SAS statistical package was used for the analyses (SAS, Version 8.2 for UNIX). Haplotype analysis was conducted based on the Stochastic-EM algorithm using THESIAS program (21). All P values are two sided.

Because polymorphism +276G→T was repeatedly associated with the risk of CVD in diabetes (12,13), we conducted a meta-analysis to summarize the associations between this polymorphism and CVD risk. Formal test of heterogeneity was assessed by a χ^2 statistic using STATA (Version 7.0; STATA, College Station, TX). We reported the summary OR derived from both the fixed-effect model and the DerSimonian and Laird random-effect model (22). In the fixed-effect model, the summary OR was obtained by averaging the natural logarithms of the ORs from individual studies, weighted by the inverses of their variances. In the random-effect model, OR was estimated by incorporating both within-study and between-study variability.

RESULTS

Baseline characteristics of study women and *ADIPOQ* genotyping. Table 1 presents the baseline characteristics in CVD case and control subjects. The case subjects were older and more obese and engaged in less physical activity than the control subjects. Among the study participants, the allele frequencies of *ADIPOQ* variants did not deviate from Hardy-Weinberg equilibrium ($P > 0.05$). The promoter polymorphism −11365C→G is not in LD with two intron 1 polymorphisms −4034A→C (D′ = 0.04 and r^2 = 0.006) and −3964A→G (D′ = 0.06 and r^2 = 0.003) but is in strong LD with the intron 2 polymorphism +276G→T (D′ = 0.8 and r^2 = 0.08), which is also in strong LD with +45T→G (D′ = 1.0 and r^2 = 0.05).

Associations with body adiposity and plasma adiponectin. Carriers of allele +45G had significantly lower BMI than the major genotypes (TT, 28.1 kg/m^2 vs. TG + GG, 26.7 kg/m^2), after adjustment for age and other covariates. Polymorphisms −11365C→G, −4034A→C,

TABLE 2
Associations of *ADIPOQ* genotypes with plasma adiponectin levels (μg/ml) in women free of CVD

SNPs	Crude			P*	Adjusted			P*
−11365C→G	CC	CG	GG		CC	CG	GG	
	7.59	7.47	5.99	0.004	7.46	7.40	6.09	0.007
−4034A→C	AA	AC	CC		AA	AC	CC	
	7.32	7.68	7.69	0.83	7.25	7.64	7.27	0.88
−3964A→G	AA	AG	GG		AA	AG	GG	
	7.33	7.82	7.81	0.52	7.33	7.64	6.76	0.23
+45T→G	TT	TG + GG	—		TT	TG + GG	—	
	7.17	8.22	—	0.01	7.27	7.84	—	0.11
+276G→T	GG	GT	TT		GG	GT	TT	
	7.23	7.68	8.07	0.21	7.26	7.65	7.95	0.15

*Except +45T→G, comparison was made between the homozygotes of the less common allele and the major genotypes; in the multivariate model, adjusting for age, BMI, smoking, alcohol consumption, physical activity, HbA$_{1c}$, history of hypertension and history of hypercholesterolemia, duration of diabetes, and postmenopausal hormone use.

TABLE 3
Associations between *ADIPOQ* genotypes and the risk of CVD

| SNPs | CVD | | OR (95% CI) | | | |
	Case subjects (%)	Control subjects (%)	Crude	P	Adjusted*	P
−11365						
CC	158 (56.6)	355 (52.0)	1.0		1.0	
CG	102 (36.6)	283 (41.5)	0.81 (0.60–1.09)	0.16	0.76 (0.55–1.04)	0.09
GG	19 (6.8)	44 (6.5)	0.97 (0.55–1.72)	0.92	0.93 (0.50–1.72)	0.81
−4034						
AA	128 (46.2)	299 (44.4)	1.0		1.0	
AC	107 (38.6)	307 (45.6)	0.81 (0.60–1.10)	0.18	0.90 (0.65–1.25)	0.52
CC	42 (15.2)	67 (10.0)	1.46 (0.94–2.27)	0.09	1.71 (1.06–2.78)	0.03
Recessive			1.62 (1.07–2.45)	0.02	1.81 (1.14–2.85)	0.01
−3964						
AA	190 (68.6)	449 (66.3)	1.0		1.0	
AG	80 (28.9)	201 (29.7)	0.94 (0.69–1.28)	0.70	1.06 (0.76–1.48)	0.74
GG	7 (2.5)	27 (4.0)	0.61 (0.26–1.43)	0.26	0.72 (0.29–1.80)	0.48
+45						
TT	204 (76.7)	529 (78.7)	1.0		1.0	
TG + GG	62 (23.3)	143 (21.3)	1.12 (0.80–1.58)	0.50	1.22 (0.84–1.76)	0.29
+276						
GG	159 (56.8)	374 (54.7)	1.0		1.0	
GT	104 (37.1)	258 (37.7)	0.95 (0.71–1.27)	0.72	0.94 (0.68–1.29)	0.70
TT	17 (6.1)	52 (7.6)	0.77 (0.43–1.37)	0.37	0.65 (0.34–1.23)	0.18

*Adjusted for age, BMI, smoking, alcohol consumption, physical activity, HbA_{1c}, history of hypertension and of hypercholesterolemia, diabetes duration, and postmenopausal hormone use.

−3964A→G, and +276G→T were not significantly associated with adiposity. In addition, +45T→G was associated with significantly higher while −11365C→G was associated with significantly lower plasma adiponectin levels (Table 2). Further adjustment for BMI and other covariates attenuated the association between +45T→G and adiponectin levels but did not appreciably change the association between −11365C→G and plasma adiponectin.

Associations with CVD risk. Polymorphism −4034A→C was significantly associated with ~60% increased CVD risk under a recessive inheritance mode (OR 1.62 [95% CI 1.07–2.45]) (Table 3). Adjustment for age, BMI, and other covariates did not appreciably change the association.

Although the frequency of the +276T homozygotes tended to be lower in the CVD case subjects, test for the association between this polymorphism and CVD risk was not statistically significant. However, haplotype analysis indicated that a common haplotype possessing allele +276T (CAATT) was associated with a significantly lower CVD risk than the most common haplotype (CAATG, 0.70 [0.50–0.98]) (Table 4).

Meta-analysis of +276G→T-CVD association. We conducted a meta-analysis to summarize the association between +276G→T and CVD risk among diabetic patients. In total, 827 CVD case and 1,887 CVD-free control subjects were included (11–13). As suggested by previous evidence, only the association under a recessive inheritance mode
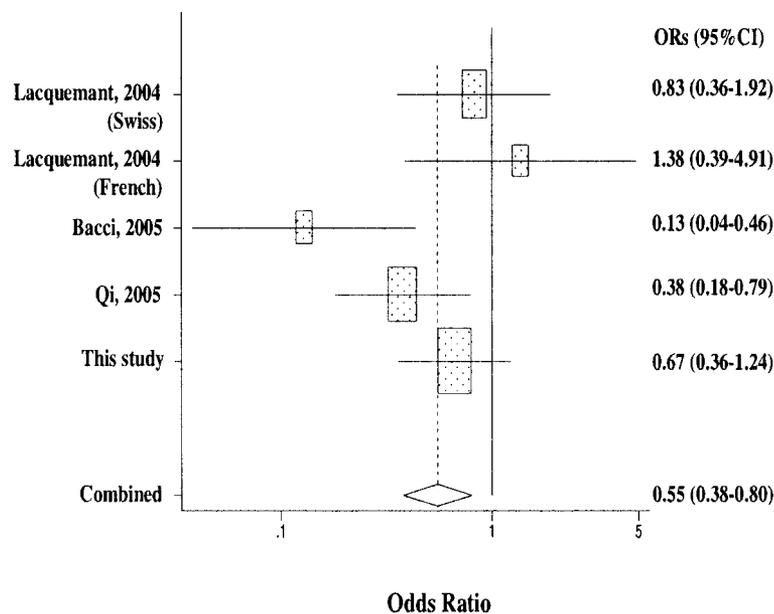

FIG. 1. Meta-analysis of the associations of adiponectin variant G276T and CVD in patients with type 2 diabetes. ORs were obtained under recessive inheritance model (GT + TT vs. GG). The studies include Bacci et al. (12), Lacquement et al. (two populations of French and Swiss) (11), Qi et al. (13), and the present study.

TABLE 4
Haplotype association with the risk of CVD in diabetic women

Haplotypes					Frequency			
−11365	−4034	−3964	+45	+276	Case subjects	Control subjects	OR (95% CI)	P
C	A	A	T	G	0.24	0.21	1.0	—
C	A	A	G	G	0.09	0.09	0.83 (0.55–1.27)	0.42
C	C	G	T	G	0.12	0.12	0.81 (0.53–1.23)	0.30
C	C	A	T	T	0.06	0.06	0.77 (0.45–1.30)	0.34
C	A	A	T	T	0.15	0.18	0.70 (0.50–0.98)	0.039
G	A	A	T	G	0.13	0.16	0.68 (0.46–1.01)	0.05

was summarized (Fig. 1). Testing for the heterogeneity of the included studies was marginally significant ($P = 0.05$). With a fixed-effect model, the +276T homozygote was significantly associated with an ~45% reduction in CVD risk (summary OR 0.55 [95% CI 0.38–0.80]). Such an association remained significant when a random-effect model was applied (0.53 [0.29–0.99]). In the analysis, by removing the initial study (11) that attributed largely to the between-study heterogeneity, the association between +276G→T and CVD risk became more significant (0.44 [0.28–0.69], fixed-effect model; test for heterogeneity, $P > 0.05$).

DISCUSSION

In this prospective study of diabetic women, the variants −11365C→G and −4034A→C in the *ADIPOQ* gene were associated with significantly decreased plasma adiponectin levels and a significantly increased cardiovascular risk, respectively. In addition, a common haplotype possessing allele +276T (CAATT) was associated with a significantly decreased CVD risk. Our meta-analysis of 827 CVD case and 1,887 CVD-free control subjects indicates that +276G→T was significantly associated with a ~45% decreased CVD risk under a recessive inheritance mode in diabetic patients.

Adiponectin is a cytokine exclusively secreted by mature adipocytes and circulates at a high concentration (1,23). Adiponectin promotes fatty acid oxidation and glucose uptake (2,3) and has shown strong anti-inflammatory and antiatherogenic effects (7,8). Plasma adiponectin levels are decreased in patients with type 2 diabetes and have been inversely associated with cardiovascular risk (5).

The finding that polymorphism −11365C→G was associated with lower plasma adiponectin levels agrees with previous evidence in French Caucasians (24). However, a recent study (25) suggests that other variants, but not −11365C→G (also coded as −11374C→G), in the *ADIPOQ* gene are responsible for adiponectin levels in an Amish population. In contrast to our findings in diabetic men (13), polymorphism +276G→T was not significantly associated with plasma adiopnectin in women, although the carriers of this polymorphism also tended to have slightly higher levels of adiponectin. Notably, +276G→T was in strong LD with −11365C→G.

In diabetic women, polymorphism −4034A→C was associated with an increased risk of CVD. Such an association was not observed in our earlier analysis in diabetic men (13) or in another study of mostly male diabetic patients (66% men; the polymorphism was also coded as −4041A→C) (11). In a test combining diabetic women and men (from the Health Professionals Follow-up Study) (13), the interaction between this polymorphism and sex was not significant (data not shown). As in diabetic men, the homozygotes of +276T also tended to be lower in the CVD case than in the control subjects among diabetic women. The haplotype analysis supports a protective effect of allele +276T on CVD risk. In addition, results from the meta-analysis confirm a significant association between +276T homozygotes and a decreased CVD risk. However, we cannot exclude the potential that +276G→T may act as a genetic marker in LD with the actual causal SNP.

We did not find a significant association between polymorphism +45T→G and CVD in diabetic women. This finding is consistent with our earlier observation in diabetic men (13) and the results from an Italian study (12), even though this polymorphism was initially associated with coronary artery disease (11). Interestingly, polymorphism +45T→G showed a strong association with body adiposity in diabetic women. Such an association is consistent with the autocrine function of adiponectin in regulating fat accumulation at adipose tissue (26). However, given its silent nature, +45T→G more likely acts as a genetic marker for other unknown causal variants.

In summary, we found that the promoter polymorphism −11365C→G in the *ADIPOQ* gene was associated with plasma adiponectin levels. Polymorphisms −4034A→C and +276G→T were associated with the risk of CVD. Our data suggest that *ADIPOQ* variability may influence plasma adiponectin levels and risk of CVD in diabetic patients. Further studies with more comprehensive and informative genetic markers are warranted to explore the genetic effects of *ADIPOQ* on CVD in our cohorts and other populations.

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