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Brief Genetics Report

The +276 Polymorphism of the *APM1* Gene, Plasma Adiponectin Concentration, and Cardiovascular Risk in Diabetic Men

Lu Qi,[1,2] Tricia Li,[1] Eric Rimm,[1,2,3] Cuilin Zhang,[1,2] Nader Rifai,[4] David Hunter,[1,2,3] Alessandro Doria,[5] and Frank B. Hu[1,2,3]

Recently, the genetic variability at adiponectin locus (*APM1*) was associated with cardiovascular risk in patients with type 2 diabetes. We sought to examine the associations of five variants of *APM1* gene (C-11365G, A-4034C, A-3964G, T45G, and G276T) with the risk of cardiovascular diseases (CVDs) in a larger cohort of diabetic patients. Of 879 diabetic men from the Health Professionals Follow-up Study, 239 participants developed coronary heart disease or stroke during 14 years of follow-up and 640 CVD-negative subjects were used as control subjects. The risk of CVD was significantly lower in TT homozygotes at locus +276 than in other genotypes under a recessive inheritance model after adjusting for age, BMI, smoking, alcohol consumption, physical activity, aspirin use, HbA_{1c}, and history of hypertension or hypercholesterolemia (odds ratio 0.38 [95% CI 0.18–0.79]; $P = 0.009$). In the CVD-negative control subjects, the allele 276T was associated with significantly higher plasma adiponectin levels in a dose-dependent pattern (GG 14.8, GT 16.2, and TT 18.8 μg/ml) after adjusting for age, BMI, and other variables (P for trend = 0.0019). In conclusion, our study showed significant associations between *APM1* G276T and decreased CVD risk and increased plasma adiponectin levels in diabetic men. *Diabetes* 54:1607–1610, 2005

Adiponectin is a newly identified cytokine that exhibits an adipose-specific expression and is abundant in blood (1). Adiponectin may promote fatty acid oxidation, glucose uptake, and insulin action in peripheral tissues such as skeletal muscle and liver (2,3). Evidence also points to an antiatherogenic property of adiponectin (4–7). In genetic studies, strong linkages have been found between the chromosomal region encompassing adiponectin gene (*APM1*) and risk factors for cardiovascular diseases (CVDs) (8,9).

Thus far, more than a dozen polymorphisms at the *APM1* locus have been explored in previous association studies (10–14). Recently, the genetic variability of *APM1* was reported to affect the risk of cardiovascular complications in diabetic patients. In one study, variant T45G (exon 2, rs2241766) was associated with an increased risk of coronary artery disease (10). In another study, the homozygosity of variant G276T (intron 2, rs1501299) was associated with a decreased risk of coronary artery disease among diabetic patients (11). However, such associations were observed in relatively small study samples (477 and 376 diabetic patients, respectively). Therefore, we sought to examine the associations of the variability of *APM1* gene and CVD risk in a larger prospective cohort of diabetic men from the Health Professionals' Follow-up Study (HPFS). We also examined the effects of the *APM1* variants on the plasma levels of adiponectin and other biomarkers of CVD.

RESEARCH DESIGN AND METHODS

The HPFS is a prospective cohort study of 51,529 American male health professionals aged 40–75 years at study initiation in 1986 (15). Information about health and disease is assessed biennially by a self-administered questionnaire. Between 1993 and 1999, 18,159 study participants provided blood samples. Among participants who returned blood samples, 999 had a confirmed diagnosis of type 2 diabetes at baseline or during follow-up through 2000. Diabetes status was confirmed using at least one of the following criteria of the National Diabetes Data Group: *1*) 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 together with at least one classic symptom (excessive thirst, polyuria, weight loss, or hunger); *2*) no symptoms but at least two elevated plasma glucose concentrations on different occasions; or *3*) taking hypoglycemic medication. We used National Diabetes Data Group criteria to define diabetes because most of our subjects were diagnosed before the release of the American Diabetes Association

From the [1]Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts; the [2]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts; the [3]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the [4]Department of Laboratory Medicine, Children's Hospital and Harvard Medical School, Boston, Massachusetts; and the [5]Research Division, Joslin Diabetes Center, Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Lu Qi, Department of Nutrition, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115. E-mail: nhlqi@channing.harvard.edu.

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CVD, cardiovascular disease; MI, myocardial infarction; SNP, single nucleotide polymorphism.

TABLE 1
Characteristics of CVD case and control subjects among diabetic men

	CVD case subjects	Control subjects	*P*
n	239	640	
Age (years)	59.6 ± 7.2	55.0 ± 8.6	<0.001
BMI (kg/m^2)	27.8 ± 4.4	27.6 ± 4.0	0.75
Alcohol intake (g/day)	8.9 ± 15.5	11.0 ± 16.4	0.27
Ethnicity*			
White	222 (97.8)	587 (95.3)	0.25
Other origins	5 (2.2)	29 (4.7)	
Current smoking*	24 (10.4)	69 (11.1)	0.80
History of hypertension	116 (48.5)	201 (31.4)	<0.001
History of hypercholesterolemia	62 (25.9)	78 (12.2)	<0.001
Current aspirin use*	102 (42.7)	192 (30.0)	<0.001
Physical activity (MET/week)*	14.3 ± 18.7	14.0 ± 17.7	0.80
Adiponectin (μg/ml)	15.6 ± 8.4	15.7 ± 8.2	0.94

Data are means ± SD and *n* (%). *Missing data were not included in calculations of frequencies.

criteria in 1997 (16). The validity of self-reported diabetes using the supplementary questionnaire had been documented previously (17).

CVD ascertainment. CVD consisted of new cases of fatal coronary heart disease, nonfatal myocardial infarction (MI), coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, fatal stroke, and nonfatal stroke that had occurred between 1986 and 2000. Nonfatal MI 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 (18), which requires evidence of a neurological deficit with sudden or rapid onset that persists for >24 h or until death. Deaths were identified from state vital statistics records and the National Death Index or reported by next of kin or the postal system. Fatal coronary heart disease was considered to have occurred if there was fatal MI confirmed by hospital records or on autopsy with the permission of the next of kin. Physicians who reviewed the records had no knowledge of the self-reported risk factors. We excluded those who had reported angina or CVD events at baseline but were not confirmed during follow-up and those who were diagnosed with CVD after the diagnosis of diabetes. A total of 879 individuals (239 CVD case and 640 control subjects) were included in this study.

Assessment of covariates, plasma adiponectin, and other biomarkers. Anthropometrical data and lifestyle factors were derived from the 1986 questionnaire. BMI was calculated as weight in kilograms divided by the square of height in meters. Physical activity was expressed as metabolic equivalent task (MET) hours based on self-reported types and durations of activities over the previous year. Plasma adiponectin was measured by competitive radioimunoassay (Linco Research, St. Charles, MO), with a coefficient of variation of 3.4%. Measurements of lipids and other biomarkers were described in detail elsewhere (7).

Genotype determination. DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Four single nucleotide polymorphisms (SNPs) (C-11365G, A-4034C, T45G, and G276T) were chosen for their ability to tag all common haplotypes at the adiponectin locus. Another SNP (A-3964G) was selected because of its lack of strong linkage disequilibrium with the four haplotype-tag SNPs (19). All SNPs were genotyped using Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). The distributions of *APM1* variants were in Hardy-Weinberg equilibrium.

Statistical analyses. A χ^2 test was used to assess fitness to Hardy-Weinberg equilibrium and to determine differences in genotype frequencies between case and control subjects. Unconditional logistic regression was used to calculate ORs. Analyses were adjusted for age, BMI, smoking, alcohol consumption, physical activity, aspirin use, HbA_{1c}, and history of hypertension or hypercholesterolemia. General linear models were used to compare geometric mean levels of biochemical measures across genotypes. Adiponectin, triglycerides, C-reactive protein, E-selectin, intracellular cell adhesion molecule-1, and vascular cell adhesion molecule-1 were logarithmically transformed. Haplotype frequencies were inferred using the expectation-maximization algorithm. The haplotypic associations were examined using the omnibus χ^2 test in SAS/genetics. All *P* values are two sided. The SAS statistical package was used for all analyses (Version 8.2 for UNIX; SAS Institute, Cary, NC).

RESULTS

Characteristics of CVD case and control subjects among the diabetic men are presented in Table 1. The CVD case subjects were older and more likely to have a history of hypertension or hypercholesterolemia than the control subjects. Mean plasma adiponectin levels were comparable between the case and control subjects.

We found a lower frequency of 276T homozygotes, which was paralleled by an increased frequency of GT heterozygotes, in the CVD case than in the control subjects (χ^2 test, $P = 0.089$). Under a recessive model, 276T homozygosity was associated with a 62% lower risk of CVD as compared with other genotypes (GG and GT) after adjusting for age, BMI, smoking, alcohol consumption, physical activity, aspirin use, HbA_{1c}, and history of hypertension or hypercholesterolemia (OR 0.38 [95% CI 0.18–0.79]; $P = 0.009$) (Table 2). The addition of plasma adiponectin, lipids, and inflammatory markers to the model did not appreciably change the association. Other polymorphisms were not associated with CVD risk. In addition, the distribution of the *APM1* haplotypes encompassing the five polymorphisms did not differ in CVD case and control subjects. Results were similar if the outcome was restricted to coronary heart disease (stroke cases were excluded, data not shown).

We also examined the associations between the *APM1* polymorphisms and plasma adiponectin, lipids, and inflammatory markers in the CVD-negative control subjects. Carriers of genotypes 276TT and GT had significantly higher adiponectin levels than GG carriers in a dose-dependent pattern after adjusting for age, BMI, and other variables (*P* for trend = 0.0019). G276T genotypes were not associated with lipids and inflammatory markers (Table 3). Other polymorphisms did not show significant associations with plasma adiponectin and other biomarkers. In addition, haplotypes driven by the allele 276T were also associated with higher plasma adiponectin levels (data not shown).

DISCUSSION

In this prospective study of diabetic men, we found that TT genotype at locus +276 of *APM1* gene was significantly associated with a lower risk of CVD. Allele 276T was also

TABLE 2
Associations between APM1 variants and the risk of CVD in diabetic men

SNP		CVD case subjects	Control subjects	OR (95% CI) Unadjusted	*P*	Adjusted*	*P*
−11365	CC	138 (59.5)	333 (53.7)	1.0		1.0	
	CG	85 (36.6)	243 (39.2)	0.85 (0.62–1.16)	0.31	0.90 (0.63–1.29)	0.58
	GG	9 (3.9)	44 (7.1)	0.50 (0.24–1.05)	0.06	0.55 (0.24–1.22)	0.14
−4034	AA	104 (44.4)	270 (43.1)	1.0		1.0	
	AC	101 (43.2)	280 (44.7)	0.94 (0.68–1.29)	0.70	0.94 (0.66–1.35)	0.76
	CC	29 (12.4)	76 (12.1)	0.99 (0.61–1.61)	0.98	1.12 (0.64–1.95)	0.68
−3964	AA	154 (66.1)	426 (68.8)	1.0		1.0	
	AG	67 (28.8)	165 (26.7)	1.13 (0.81–1.59)	0.46	1.24 (0.85–1.83)	0.26
	GG	12 (5.1)	28 (4.5)	1.20 (0.59–2.41)	0.61	0.98 (0.43–2.26)	0.96
+45	TT	170 (77.6)	440 (73.5)	1.0		1.0	
	TG + GG	49 (22.4)	159 (26.5)	0.80 (0.55–1.15)	0.22	0.77 (0.51–1.16)	0.21
+276	GG	105 (46.0)	293 (49.3)	1.0		1.0	
	GT	111 (48.7)	249 (41.9)	1.30 (0.93–1.77)	0.10	1.33 (0.94–1.89)	0.11
	TT	12 (5.3)	52 (8.8)	0.67 (0.35–1.31)	0.24	0.46 (0.22–0.97)	0.04
	TT vs. GG + GT			0.58 (0.30–1.11)	0.08	0.38 (0.18–0.79)	0.009

Data are *n* (%) unless otherwise indicated. *ORs were estimated adjusting for age, BMI, smoking, alcohol consumption, physical activity, HbA_{1c}, aspirin use, history of hypertension, and history of hypercholesterolemia.

associated with higher adiponectin levels in a dose-dependent pattern. However, the association between G276T and CVD risk appears to be independent of plasma adiponectin level.

Adiponectin is a recently identified adipose-specific cytokine that is abundant in blood. Adiponectin plays pivotal roles in the regulation of insulin action and metabolisms of glucose and fatty acid (2–4). Accumulating evidences indicate that adiponectin may also have antiinflammatory and antiatherogenic properties (5–7). In addition, genetic data support a strong linkage between the chromosome region encompassing *APM1* gene and risk factors for CVD (8,9). A protective effect of 276T homozygosis against coronary artery disease was recently found in diabetic patients from Italy (adjusted OR 0.13 [95% CI 0.037–0.46]) (11). Similar to our findings, the Italian study also found that the case subjects had a higher frequency of GT heterozygotes than the control subjects. The reason for this finding is unclear but may be due to chance.

TABLE 3
Associations between G276T and biochemical measures in diabetic men without CVD

	Genotypes GG	GT	TT	*P* value*
n	293	249	52	
Lipids				
Total cholesterol (mg/dl)	212	206	205	0.22
LDL (mg/dl)	126	122	121	0.33
HDL (mg/dl)	41.3	41.2	41.3	0.99
Triglycerides (mg/dl)†	185	176	162	0.25
Inflammatory markers				
TNF R2 (pg/ml)	2,900	2,918	2,971	0.90
CRP (mg/dl)	0.32	0.27	0.26	0.28
Endothelial biomarkers				
ICAM-1 (ng/ml)	355	357	348	0.83
VCAM-1 (ng/ml)	1,369	1,404	1,372	0.79
Adiponectin (μg/ml)	14.8	16.2	18.8	0.0029

Data are means. **P* value obtained in ANOVA test in the comparison across G276T genotypes (additive model) adjusting for age, BMI, smoking, alcohol consumption, physical activity, HbA_{1c}, aspirin use, history of hypertension, and history of hypercholesterolemia. †Only in subjects who fasted before blood draw, $n = 331$. CRP, C-reactive protein; ICAM-1, intracellular cell adhesion molecule-1; TNF R2, tumor necrosis factor receptor II; VCAM-1, vascular cell adhesion molecule-1.

In a previous study (20), we reported that plasma adiponectin levels were associated with improved glycemic control, better lipid profile, and reduced inflammation in diabetic men. Therefore, we hypothesized that the protective effects of 276T on CVD might be mediated by plasma adiponectin. We observed that allele 276T was associated with increased plasma adiponectin in the CVD-negative control subjects. The adiponectin elevation effects of 276T have been found in some (14) but not all (13) previous studies among nondiabetic subjects. However, adjustment for adiponectin level did not affect the association between G276T and CVD risk. Also in the Italian study (11), circulating adiponectin did not appear to mediate the genetic effect. This may be explained in part by the relative inaccuracy of plasma adiponectin level in representing adiponectin concentration at its action sites, e.g., subendothelial space. The effects of G276T on CVD risk were also independent of biomarkers related to lipid metabolism, inflammation, and endothelial function in our study.

Lacquemant et al. (10) recently reported that variant T45G was associated with an increased risk of coronary artery disease among diabetic patients. However, such an association was not observed in our study or in the Italian study (11). The reason for the discrepancies among these studies is unclear. Considering that replication is the fundamental tool to validate genetic findings, more studies are warranted to examine the effects of T45G on cardiovascular events in diabetic patients.

As a potential limitation, population stratification may influence the observed associations. However, our population was racially homogeneous, with the majority of the participants being white (96%). Further adjustment for ethnicity or removing the minorities from the analyses did not change our results.

In summary, we found that the homozygous allele T at +276 of *APM1* gene was associated with a significantly lower risk of CVD in diabetic men. Allele 276T was also associated with an increased level of plasma adiponectin. However, the protective effects of 276T appear to be independent of the circulating adiponectin and other markers related to lipids, inflammation, and endothelial function. The mechanisms underlying the association between G276T and CVD risk need to be investigated in future studies.

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