Genetic Variation at the Adiponectin Locus and Risk of Type 2 Diabetes in Women

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Previous data suggesting that polymorphisms in the adiponectin gene were associated with insulin resistance or type 2 diabetes have been inconsistent. We assessed the relationship between five common haplotype-tagging single nucleotide polymorphisms (SNPs) in the adiponectin gene (−11365C>G, −4034A>C, −3964A>G, +45T>G, and +276G>T), haplotypes defined by these SNPs, and the risk of type 2 diabetes by conducting a nested case-control study of 642 incident cases of type 2 diabetes and 995 matching control subjects in the Nurses' Health Study. Overall, we did not observe significant differences in genotype or allele frequencies for the five SNPs between the case and control subjects. After adjustment for diabetes risk factors, the −4034 C/C genotype was associated with a reduced risk of diabetes (odds ratio [OR] compared with the A/A genotype = 0.70, 95% CI 0.50–0.99, $P = 0.04$). In subgroup analyses, the +276 genotype was significantly associated with diabetes risk only among subjects with peroxisome proliferator–activated receptor-γ (*PPARγ*) variant 12Ala allele (OR comparing +276 T alleles with the G/G genotype = 1.69, 1.04–2.75, $P = 0.035$) or among obese subjects (1.46, 1.03–2.08, $P = 0.03$). These data suggest a potential interaction between the adiponectin genotype and *PPARγ* genotype or obesity, but these analyses should be considered exploratory and require further investigation in larger studies. *Diabetes* 53:209–213, 2004

Adiponectin (also known as APM1, Acrp30, or adipoQ) is a circulating protein that shares significant similarities with collagens VIII and X and complement protein C1q (1,2). Humans with obesity, insulin resistance, or type 2 diabetes have lower plasma levels of adiponectin than normal control subjects (3). Rosiglitazone, a peroxisome proliferator–activated receptor-γ (PPARγ) agonist and insulin-sensitizing agent, has been shown to increase plasma adiponectin levels in both diabetic and nondiabetic subjects, suggesting a role of PPARγ agonism in the modulation of adiponectin gene transcription (4).

The adiponectin gene localizes to human chromosome 3q27 (5), a region identified as a susceptibility locus for the metabolic syndrome and type 2 diabetes in Caucasians (6). Screening for this gene in Japanese (7) and Caucasian populations (8) has uncovered >10 single nucleotide polymorphisms (SNPs). Two of the SNPs (+45 and +276) were significantly associated with risk of type 2 diabetes in a Japanese study (7) but not in a French population (8). In a German study (9), the +45 SNP was significantly associated with obesity and insulin resistance among subjects with no family history of diabetes. In a recent study (10), a haplotype defined by the +45 and +276 SNPs was significantly associated with obesity and insulin resistance.

We conducted a prospective nested case-control study of genetic variation in the adiponectin gene and risk of type 2 diabetes in the Nurses' Health Study. We also tested a potential interaction between adiponectin genotypes and *PPARγ* genotype based on PPARγ's role in the regulation of adiponectin production and because the *PPARγ* Pro12Ala polymorphism has been associated with lower risk of type 2 diabetes (11).

RESEARCH DESIGN AND METHODS

The details of the Nurses' Health Study have been reported elsewhere (12). Briefly, the cohort was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on their medical history and lifestyle. Every 2 years, participant lifestyle factors, including smoking, menopausal status and postmenopausal hormone therapy, and body weight, have been updated by validated questionnaires. Reported weights have been shown to correlate with measured weights ($r = 0.96$) (13).

Samples for the present case-control study were selected from a subcohort of 32,826 women who provided a blood sample between 1989 and 1990 and were free from diabetes, cardiovascular disease, stroke, or cancer at the time of blood collection. Incident cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire and diagnosed at least 1 year after blood collection through 2000. The supplementary questionnaire obtained information on symptoms, diagnostic tests, and hypoglycemic therapy used to define type 2 diabetes cases. Medical record review confirmed the diagnosis of type 2 diabetes using this questionnaire for 98% of cases using the National Diabetes Data Group criteria (14), verifying the validity of this method (15). We used the American Diabetes Association diagnostic criteria (16) for diagnosis of diabetes cases during the 1998 and 2000 cycles.

There were 642 incident cases diagnosed at least 1 year after blood collection through 2000 that were matched to 995 control subjects who did not report physician-diagnosed diabetes. For the cases diagnosed in 1996 or earlier, two control subjects were matched to each case subject for age, month and year of blood draw, and fasting status at blood draw. One of the

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PPARγ, peroxisome proliferator–activated receptor-γ; SNP, single nucleotide polymorphism.

two control subjects was also matched according to BMI (±1 kg/m^2). For the cases diagnosed after 1996, one control subject was matched to each case subject for age, month and year of blood draw, and fasting status, and an additional control subject was also matched for BMI for cases in the top BMI decile, for better control of obesity.

DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp Blood Kit (Qiagen, Chatsworth, CA). Four SNPs (−11365C>G, −4034A>C, +45T>G, and +276G>T) were chosen for genotyping based on their ability to tag all common haplotypes at the adiponectin locus; these common haplotypes (frequency >5%) account for >70% of the haplotypes at this locus (10). Another SNP (−3964A>G) was selected because of its lack of strong linkage disequilibrium with the four haplotype-tagging SNPs. The primary genotyping technique was Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). Genotype frequencies for all SNPs were found to be in Hardy-Weinberg equilibrium ($P >$ 0.05).

Plasma insulin, C-peptide, and proinsulin were determined by radioimmunoassay using a commercial kit (Linco Research, St. Charles, MO). Ten percent of the samples assayed were redundant samples included for quality control. Within-individual coefficients of variation among the redundant samples were 13.9, 6.9, and 7.3% for insulin, C-peptide, and proinsulin, respectively.

Genotype and allele frequencies between case and control subjects were compared using χ^2 tests. Odds ratios (ORs) were determined using unconditional multivariate logistic regression adjusting for type 2 diabetes risk factors assessed in the 1988 cycle, including age (5-year categories), physical activity (<1.5, 1.5–5.9, 6.0–11.9, 12–20.9, and ≥21.0 metabolic equivalent hours/week), smoking (never, past, and current [<15, 15–24, or ≥25 cigarettes/day]), alcohol intake (nondrinker and drinker [0.1–4.9, 5–10, or >10 g/day]), and BMI (<23, 23–24.9, 25–29.9, 30–34.9, or ≥35 kg/m^2). Interactions between adiponectin genotypes and *PPARγ* Pro12Ala genotype (Pro/Pro, Pro/Ala+Ala/Ala) and obesity (BMI <30, ≥30 kg/m^2) were assessed using a likelihood ratio test.

Haplotype frequencies were estimated by SAS/Genetics using the expectation-maximization algorithm (17). We used χ^2 tests to examine whether overall haplotype frequencies were significantly different between case and control subjects. These analyses were repeated for strata defined by *PPARγ* Pro12Ala genotype (Pro/Pro, Pro/Ala+Ala/Ala) and obesity (BMI <30, ≥30 kg/m^2).

RESULTS

Characteristics of the case and control subjects are presented in Table 1. The baseline plasma concentrations of fasting insulin, C-peptide, and proinsulin were significantly higher in case than control subjects.

Overall, there were no statistically significant differences in the distribution of genotypes comparing case with control subjects (Table 2). Individual SNPs at positions +45 and +276 were not associated with risk of diabetes. The haplotype defined by these two SNPs was also not associated with diabetes risk (data not shown). For the −4034 SNP, there was a suggestion of lower diabetes risk among subjects with the C/C genotype compared with those with the A/A genotype (OR = 0.70, 95% CI 0.50–0.99, P = 0.04), but the A/C genotype was not associated with diabetes risk. The OR of diabetes comparing subjects with the C/C genotype with those with the A allele was 0.77 (0.56–1.04).

In stratified analyses (Table 3), subjects with the T allele (G/T or T/T genotype) at position +276 had a significantly increased risk of type 2 diabetes compared with subjects with G/G genotype among subjects with the *PPARγ* alanine allele (OR = 1.69, 95% CI 1.04–2.75, P = 0.035), but not among subjects with *PPARγ* proline allele (P for interaction = 0.14). Women with both the +276 G/G genotype and the *PPARγ* alanine allele had a lower risk of diabetes (0.56, 0.38–0.83) compared with women with both the adiponectin +276 T allele and the PPARγ Pro/Pro genotype. Likewise, among obese subjects the +276 T allele was associated with increased risk of diabetes compared with those with the G/G genotype (1.46, 1.03–2.08, P = 0.03), but this association was not observed among nonobese subjects (P for interaction = 0.10). Adjustment for PPARγ genotype did not affect these results.

TABLE 1
A comparison of diabetes risk factors between case and control subjects*

	Case subjects	Control subjects	P
n	642	995	
Age (years)	54.51 ± 6.83	54.40 ± 6.81	0.74
BMI (kg/m^2)	30.45 ± 5.62	27.22 ± 5.78	<0.001
Physical activity (metabolic equivalent hours/week)	12.40 ± 15.31	14.97 ± 17.98	0.002
Alcohol intake (g/day)	3.68 ± 6.93	6.07 ± 9.30	<0.001
Waist circumference (in)	35.48 ± 4.94	32.13 ± 4.87	<0.001
Ethnicity (Caucasian)	94.8	95.8	0.39
Current smoker	13.9	12.4	0.38
Postmenopausal status	75.7	74.1	0.46
Family history of diabetes (first-degree relatives)	47.5	22.1	<0.001
History of hypertension	48.6	28.6	<0.001
History of high cholesterol	32.4	24.5	0.0005
Biomarkers†			
Insulin (μU/ml)	13.67 ± 9.08	10.55 ± 6.22	<0.001
C-peptide (pm/ml)	0.99 ± 0.70	0.61 ± 0.40	<0.001
Proinsulin (fmol/ml)	26.51 ± 22.14	11.69 ± 8.88	<0.001

Data are means ± SD or percent. *Case and control subjects were matched for age, fasting status, and race. †The biomarker analyses included 432 case and 398 control subjects.

Table 4 presents the frequencies of the six common haplotypes defined by the five SNPs for all case and control subjects and for subjects with the *PPARγ* alanine allele or obesity. Overall, there were no significant differences in haplotype frequencies between case and control subjects (P = 0.51). When stratified by *PPARγ* Pro12Ala genotype or obesity, there were no significant differences in overall haplotype frequencies between case and control subjects for PPARγ 12Pro allele carriers or nonobese subjects (data not shown). There was a suggestion of an overall difference in hapolotype frequencies between case and control subjects among obese subjects (P = 0.06). The difference was primarily due to excess frequency for the C-A-G-T-G haplotype (a subhaplotype of the +45/+276 T-G haplotype) and lower frequency for the G-A-A-T-T haplotype in case subjects compared with the control subjects. However, tests for the differences for these individual haplotypes between case and control subjects did not reach statistical significance (data not shown).

Among the control subjects, none of the individual SNPs in the adiponectin gene were significantly associated with variation in levels of fasting insulin, C-peptide, or proinsulin (data not shown). The prevalence of obesity (BMI ≥30 kg/m^2) was higher among carriers of the +45 T allele compared with the G allele (31 vs. 23%, P = 0.047), and the haplotype defined by +45 T and +276 G was associated with a significantly higher prevalence of obesity (35 vs. 27%, P = 0.027). However, this haplotype was not associ-

TABLE 2
Associations between SNPs in adiponectin gene and risk of type 2 diabetes

		Case subjects	Control subjects	Unadjusted OR	Multivariate OR*
SNP-11365	C/C	357 (55.6)	557 (56.0)	1.0	1.0
	C/G	244 (38.0)	379 (38.1)	1.00 (0.81–1.23)	1.01 (0.81–1.27)
	G/G	41 (6.4)	59 (5.9)	1.08 (0.71–1.64)	1.02 (0.65–1.59)
SNP-4034	A/A	292 (45.5)	418 (42.0)	1.0	1.0
	A/C	280 (43.6)	439 (44.1)	0.92 (0.74–1.13)	0.96 (0.76–1.20)
	C/C	70 (10.9)	138 (13.9)	0.73 (0.53–1.01)	0.70 (0.50–0.99)
SNP-3964	A/A	440 (68.5)	633 (63.6)	1.0	1.0
	A/G	177 (27.6)	331 (33.3)	0.78 (0.63–0.97)	0.79 (0.62–1.00)
	G/G	25 (3.9)	31 (3.1)	1.16 (0.68–2.00)	1.20 (0.67–2.13)
SNP + 45	T/T	518 (80.7)	785 (78.9)	1.0	1.0
	T/G + G/G	124 (19.3)	210 (21.1)	0.90 (0.70–1.15)	0.91 (0.70–1.19)
SNP + 276	G/G	322 (50.2)	523 (52.6)	1.0	1.0
	G/T	266 (41.4)	399 (40.1)	1.08 (0.88–1.34)	1.16 (0.93–1.45)
	T/T	54 (8.4)	73 (7.3)	1.20 (0.82–1.75)	1.22 (0.81–1.82)

Data are *n* (%) or OR (95% CI). *Adjusted for age (5-year categories), alcohol consumption (nondrinkers, 0–4.9, 5–10, or >10 g/day), physical activity (quintiles of metabolic equivalent hours/week), smoking (never, past, and current smoker of 1–14, 15–24, and ≥25 cigarettes/day), and BMI (<23, 23–24.9, 25–29.9, 30–34.9, and ≥35 kg/m^2).

ated with elevated fasting insulin or C-peptide levels. Subjects with the PPARγ 12Ala allele and the adiponectin +276 G/G allele, who had a significantly lower risk of type 2 diabetes compared with subjects with the PPARγ Pro allele and the adiponectin +276 C allele, had slightly lower levels of fasting insulin (12.0 vs. 14.1 μU/ml, $P = 0.06$).

DISCUSSION

Our data do not suggest an important role of adiponectin SNPs or haplotypes in the development of type 2 diabetes in Caucasian women. Subgroup analyses suggest a significant association between SNP +276 and risk of diabetes among PPARγ alanine allele carriers or among obese subjects. Although biologically plausible, these interaction tests had limited power and should be considered exploratory.

Prior data on adiponectin gene variation and risk of type 2 diabetes have been inconsistent. In a Japanese study (7) that included 384 type 2 diabetic subjects and 480 nondiabetic control subjects, subjects with the G/G genotype at position +45 or the G/G genotype at position +276 had a significantly increased risk of diabetes compared with those with the T/T genotype at these loci. In a study conducted in Germany (9), subjects with G/G + G/T genotypes at position +45 had higher BMI and lower insulin sensitivity, but these associations were significant only among subjects without a family history of diabetes, not among those with a family history of diabetes. Recently, Menzaghi et al. (10) reported that a haplotype defined by the +45 and +276 SNPs was associated with several components of the insulin resistance syndrome. However, neither the two individual SNPs nor their haplotype were associated with risk of type 2 diabetes. Similarly, a study conducted in French Caucasian families (8) did not detect any significant associations between

TABLE 3
The association between adiponectin +276 and risk of type 2 diabetes according to PPARγ genotype and obesity

	Case subjects	Control subjects	OR (95% CI)	*P* for interaction
PPARγ Pro/Pro				
SNP +276				
G/G	269 (52.2)	392 (52.6)	1.0*	
G/T + T/T	246 (47.8)	354 (47.5)	1.09 (0.86–1.39)*	0.14
PPARγ Ala				
SNP +276				
G/G	51 (40.8)	127 (52.1)	1.0*	
G/T + T/T	74 (59.2)	117 (48.0)	1.69 (1.04–2.75)*	
BMI <30 kg/m^2				
SNP +276				
G/G	172 (51.5)	372 (51.5)	1.0†	
G/T + T/T	161 (48.4)	350 (48.5)	1.04 (0.79–1.36)†	0.10
BMI ≥30 kg/m^2				
SNP +276				
G/G	143 (48.6)	143 (56.1)	1.0†	
G/T + T/T	151 (51.4)	112 (43.9)	1.46 (1.03–1.08)†	

Data are *n* (%) unless otherwise indicated. The number of case and control subjects in these analyses was less than the overall study due to missing data on PPARγ genotype or obesity. *Adjusted for age (5-year categories), alcohol consumption (nondrinkers, 0–4.9, 5–10, or ≥10 g/day), physical activity (quintiles of metabolic equivalent hours/week), smoking (never, past, and current smoker of 1–14, 15–24, and ≥25 cigarettes/day), and BMI (<23, 23–24.9, 25–29.9, 30–34.9, and ≥35 kg/m^2). †Adjusted for age (5-year categories), alcohol consumption (nondrinkers, 0–4.9, 5–10, or ≥10 g/day), physical activity (quintiles of metabolic equivalent hours/week), and smoking (never, past, and current smoker of 1–14, 15–24, and ≥25 cigarettes/day).

TABLE 4
Haplotype frequency difference between case and control subjects for all subjects, subjects with the PPARγ12 Ala allele, and obese subjects

Inferred haplotypes					Total		PPARγ 12 Ala allele		Obese subjects	
					Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects
−11365	−4034	−3964	+45	+276	(642 × 2 = 1284)*	(995 × 2 = 1990)	(125 × 2 = 250)	(244 × 2 = 488)	(286 × 2 = 572)	(241 × 2 = 482)
C	A	A	T	G	21%	21%	23%	20%	21%	24%
C	A	G	T	G	20%	19%	19%	17%	21%	16%
C	A	A	G	G	16%	15%	16%	15%	16%	14%
G	A	A	T	T	12%	13%	10%	13%	10%	15%
C	C	A	T	G	9%	8%	8%	11%	9%	7%
C	A	G	T	T	5%	5%	11%	7%	7%	7%
All others					17%	19%	13%	17%	16%	17%
P					0.51		0.11		0.06	

*The number of chromosomes = *n* subjects × 2.

these two SNPs and risk of type 2 diabetes, although it suggested a possible association with two other SNPs (−11391G>A and −11377C>G).

Consistent with the analysis by Menzaghi et al. (10), we found that the +45/+276 haplotype was associated with a greater prevalence of obesity. However, we did not observe an association between the haplotype and fasting insulin levels. These results suggest that the +45 or +276 SNPs in the adiponectin gene are unlikely to play a major role in the development of diabetes in Caucasians.

Our study suggests a possible interaction between SNPs in the adiponectin and *PPARγ* genes on diabetes risk. We found that subjects with the 12Ala allele and the adiponectin +276 G/G allele had a lower risk of type 2 diabetes. Although the exact biological basis for the finding is unclear, this combination appeared to be associated with somewhat lower fasting insulin levels among control subjects. One limitation of our study is that we did not measure plasma levels of adiponectin.

In conclusion, we did not detect important associations between the five individual SNPs in the adiponectin gene, or common haplotypes defined by these SNPs, and risk of type 2 diabetes. There was a suggestion of interaction between the +276 genotype and the *PPARγ* Pro12Ala polymorphism or obesity on risk of type 2 diabetes, but these results were not a primary aim of the analysis, need to be interpreted with caution, and should be replicated in future studies with much larger samples. Although adiponectin protein has consistent and robust associations with many metabolic and diabetes traits, the biological effects of this protein are not evidently mediated through genetic mechanisms.

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