CLINICAL STUDY

Association of the +45T>G and +276G>T polymorphisms in the adiponectin gene with insulin resistance in nondiabetic Greek women

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Abstract

Objective: We explored potential associations of two single nucleotide polymorphisms (SNPs) in the adiponectin gene (*ADIPOQ*; +45T>G, rs2241766 and +276G>T, rs1501299) with circulating total and high-molecular weight (HMW) adiponectin, insulin resistance (IR), and markers of obesity in a healthy Greek female population.

Design and methods: The two SNPs were genotyped in 349 women without diabetes (mean age: 47.0±12.1 years, mean body mass index: 28.9±5.6 kg/m²). Total and HMW adiponectin concentrations, body composition variables, IR parameters, and plasma lipid levels were determined.

Results: In single SNP analysis adjusting for several potential confounders, SNP +276G>T was associated with higher fasting insulin levels ($P=0.01$) and higher homeostasis model assessment index for IR (HOMA-IR; $P=0.009$), and SNP +45T>G was associated with lower insulin levels and HOMA-IR ($P=0.05$ and $P=0.07$ respectively). No association with total or HMW adiponectin, plasma lipid levels, and body composition variables was observed; however, haplotype analysis revealed that subjects homozygous for the most common +45T/+276G haplotype had lower total adiponectin levels than did noncarriers of this haplotype ($P=0.02$). The observed differences in HOMA-IR were very significant among women with a higher body fat (BF) percentage ($\geq$ the population median of 41%; all $P\leq 0.005$), but not among leaner individuals (P for interactions 0.01–0.07), thus suggesting that *ADIPOQ* effects on insulin sensitivity may depend upon BF status.

Conclusion: Our data suggest a significant role of *ADIPOQ* variants at positions +45 and +276 in the development of IR in healthy Greek women possibly through an interaction with BF.

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Introduction

Adiponectin is specifically and abundantly expressed in adipocytes and circulates in the blood at high concentrations (1). The evidence from animal models and human studies supports an important role for adiponectin in the pathophysiology of metabolic syndrome and most of its individual components, namely insulin resistance (IR), obesity, and dyslipidemia (2, 3). Concentrations of adiponectin are reduced in persons who are obese compared with lean individuals (4), and prospective studies have consistently found a decreased risk for type 2 diabetes mellitus (T2DM) with increasing concentrations of total adiponectin (5–7). Adiponectin circulates in plasma as a trimer, hexamer, and a high-molecular weight (HMW) form (4, 5). There is considerable evidence that the HMW multimer is the active form of the hormone and it has been proposed

to be a better predictor of IR, metabolic syndrome, or cardiovascular disease (CVD) than total adiponectin (8, 9). Recently, it was shown that the ratio of HMW to total adiponectin is related to risk for T2DM independent of total adiponectin, suggesting an important role of the relative proportion of HMW adiponectin in diabetes pathogenesis (7).

Several adiponectin gene (*ADIPOQ*) single nucleotide polymorphisms (SNPs) have been shown to influence adiponectin levels and have been associated with risk for obesity, IR, T2DM, and CVD (10–17). Two of the most commonly studied SNPs at the *ADIPOQ* locus are a silent T to G substitution in exon 2 (+45T>G, rs2241766) and a G to T substitution in intron 2 (+276G>T, rs1501299). However, association studies of these two SNPs, either independently or as a haplotype, have resulted in conflicting evidence in different populations and sample types (18). Stumvoll *et al.* reported a positive

association between the minor +45G allele of the +45T>G polymorphism and obesity traits in a healthy German population (11), whereas in Taiwanese non-diabetic subjects the same allele was related to a lower risk of obesity (19). Recently, the presence of the +45G allele was associated with increased risk of IR in a sample of Greek women with polycystic ovary syndrome (20) and a Spanish population (21), while in a Chinese/Japanese sample it was the +45T allele that presented such an association (19). The +276G allele has been associated either with increased or decreased levels of plasma total adiponectin in different Caucasian populations (22, 23), while this allele has been positively associated with obesity in Sweden (12), but not in Finland (24). Reverse associations with IR were also observed for SNP +276G>T in Italian (25) versus Japanese (8) and Polish populations (26). Haplotypes comprised of the two SNPs have also provided evidence of association with plasma total adiponectin levels, obesity, and IR phenotypes (10, 27, 28); however, little is known regarding the effect of these two SNPs on circulating HMW adiponectin, either independently or at the haplotypic level. So far, there is only one study evaluating the variation of HMW adiponectin levels according to SNP +276G>T genotypes in Japanese populations (29).

The aim of the present study was to examine the association of SNPs +45 and +276 in the *ADIPOQ* gene with markers of obesity, circulating total and HMW adiponectin concentrations, and IR parameters in a group of Greek women without diabetes.

Subjects and methods

Subjects

A total of 379 Greek white women without a known history of diabetes, CVD, or cancer were enrolled in this study, which was approved by the Institutional Review Board of Harokopio University. Data on general health status, smoking habits, and present medications, including hormone replacement and oral contraceptive treatment, were collected using an interviewer-administered questionnaire. Menstruation status was also recorded; women were classified as premenopausal if they had regular menses, perimenopausal if they were suffering from irregular menses, and postmenopausal if they had ceased menstruating for at least 12 months. After giving written informed consent to participate, subjects provided a fasting blood sample, and underwent anthropometric and body composition measurements. Fasting glucose concentrations >126 mg/dl, cortisol treatment, and lipid-lowering medication were criteria for exclusion from the analysis. Thus, our analysis was restricted to 349 apparently healthy women without diabetes. Of those, eight were on hormone replacement therapy and three reported regular use of oral contraceptives.

Anthropometry and body composition

Anthropometric and body composition measurements were performed with the subject wearing light clothing and without shoes. For all subjects, body weight and height were measured using a scale and a wall-mounted stadiometer to the nearest 0.5 kg and 0.5 cm respectively. Body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared. Waist circumference (cm) was measured in the middle between the 12th rib and the iliac crest, and hip circumference (cm) was measured around the buttocks, at the level of the maximum extension. The waist-to-hip ratio was then calculated.

Dual X-ray absorptiometry (DXA) was used as a reference method for the assessment of body composition. Soft tissue composition was determined using a DXA total body scanner (Model DPX+, Lunar Corp., Madison, WI, USA) and the Lunar software 4.7e. Anthropometric and body composition measurements were performed in all study participants by two trained investigators (L M and M K).

Biochemical analysis

Blood samples were drawn after a 12-h fast between 0830 and 1030 h, and plasma was immediately frozen in -80°C until biochemical analysis. Fasting plasma glucose concentrations, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were determined using commercially available enzymatic colorimetric assays (Alfa Wassermann BV, Woerden, The Netherlands) on an automated ACE analyzer (Schiapparelli Biosystems, Inc, Fairfield, New Jersey, USA). Within-batch coefficients of variation (CV) for the determination of these biochemical variables were all below 5%. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation (30). Apolipoprotein (apo)A1 and apoB were determined by turbidometry at 340 nm using a specific antiserum (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). Owing to insufficient plasma samples, apoA1 and apoB levels were not available for ten subjects.

Fasting plasma total adiponectin and insulin concentrations were measured by RIA as follows: adiponectin (Linco Research, St Charles, MO, USA; sensitivity, 1 ng/ml; intraassay CV, 1.78–6.21%); insulin (Diagnostics Systems Laboratory, Webster, TX, USA; sensitivity, 1.3 $\mu\text{IU/ml}$; intraassay CV, 4.5–8.3%). In addition, plasma levels of HMW adiponectin were determined in a subgroup of the study participants using a novel ELISA test (Human Multimeric Adiponectin ELISA; ALPCO Diagnostics, Salem, NH, USA). The sensitivity of this assay was 0.05 ng/ml. IR was estimated using the homeostasis model assessment (HOMA) with the following formula: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/ml}) \times \text{fasting glucose } (\text{mmol/l}) / 22.5$ (31).

Genotype determination

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood using a standard salting-out procedure. Polymorphisms +45T>G (rs2241766) and +276G>T (rs1501299) were genotyped on a 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA, USA) using fluorescent allelic discrimination TaqMan assays. Genotyping was performed at the Laboratory of Nutrition and Genomics, JM-USDA-HNRCA at Tufts University, Boston. Because of PCR failure, genotypes could not be determined for seven individuals at position +45 and 15 individuals at position +276. Quality control evaluations were regularly taken for all laboratory procedures.

Statistical analysis

To test whether each of the SNPs was in Hardy–Weinberg equilibrium and investigate the strength of pairwise linkage disequilibrium (LD) between SNPs (expressed as D'), we used the Genetic Data Analysis software HelixTree (Golden Helix, Inc., Bozeman, MT, USA). This package was also used to estimate haplotype frequencies of the most common haplotypes (frequency >1%) using the expectation-maximization algorithm (32). Allele frequencies were determined with the gene counting method. All other statistical analyses were carried out using SPSS software package (version 13.0, SPSS, Chicago, IL, USA). Data were log transformed when necessary to approximate a normal distribution. Pearson correlation coefficients were calculated to describe associations of plasma total and HMW adiponectin, and HOMA-IR with anthropometric and blood measures. To evaluate the association between adiponectin gene polymorphisms (individually or as a haplotype) and continuous variables, we performed analysis-of-covariance (ANCOVA) using a general linear model with Bonferroni *post-hoc* comparisons. Multiple regression analysis was also applied where appropriate. Models were adjusted for the following potential confounders: age, estrogen use, smoking status, menopausal status, and percentage body fat (BF) or BMI. Both codominant and dominant models for the minor alleles were evaluated. The statistical homogeneity of the effects by BF categories was tested using interaction terms. All P values presented are two tailed and were considered significant if they were <0.05.

Results

Sample characteristics and frequencies of +45T>G and +276G>T SNPs, and +45/+276 haplotypes

The general characteristics of the 349 Greek nondiabetic women participating in the study are presented in Table 1. The mean age was 47.0 ± 12.1 years

Table 1 General characteristics of study participants ($n=349$).

Demographic, lifestyle, and clinical parameters	
Age (years)	47.0 (45.7–48.2)
Weight (kg)	73.9 (72.3–75.4)
Body mass index (kg/m ²)	28.9 (28.4–29.5)
Body fat (kg)	30.8 (29.9–32.1)
Body fat (%)	41.0 (40.3–41.7)
Waist circumference (cm)	85.8 (84.6–87.1)
Waist-to-hip ratio	0.79 (0.78–0.79)
Obesity (normal weight/overweight/obese) (%)	23.2/42.1/34.7
Smoking status (current smokers/past/never) (%)	37.0/7.7/55.3
Menopausal status (premenopausal/peri/post) (%)	47.0/8.9/44.1
Hormonal and metabolic parameters	
Total adiponectin (µg/ml)	14.56 (13.64–15.15)
HMW adiponectin (µg/ml)	5.69 (5.18–6.21)
Fasting insulin (µIU/ml)	8.71 (8.21–9.21)
Fasting glucose (mg/dl)	95.1 (93.9–96.2)
Insulin resistance (HOMA-IR)	2.07 (1.94–2.19)
Triglycerides (mg/dl)	95.6 (90.5–100.6)
Total cholesterol (mg/dl)	218.9 (214.3–223.5)
LDL cholesterol (mg/dl)	147.9 (144.1–151.6)
HDL cholesterol (mg/dl)	51.8 (50.7–52.9)
Total cholesterol/HDL	4.33 (4.24–4.43)
ApoA1 (mg/dl)	128.5 (126.2–130.9)
ApoB (mg/dl)	85.7 (83.4–88.1)

Data are presented as mean ($\pm$ 95% CI) for continuous variables or % for categorical variables. Normal weight: BMI <25 kg/m²; overweight: 25 kg/m² $\leq$ BMI <30 kg/m²; obese: BMI $\geq$ 30 kg/m². HMW adiponectin, high-molecular weight adiponectin; available for 211 subjects. Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) with the following formula: HOMA-IR = fasting insulin (µIU/ml) $\times$ fasting glucose (mmol/l)/22.5. CI, confidence intervals.

(range: 18–74 years), and the mean BMI was 28.9 ± 5.6 kg/m² (range: 18.5–47.7 kg/m²). A high proportion of women were overweight and obese (42.1 and 34.7% respectively).

The genotype distributions of the +45T>G and +276G>T SNPs in the adiponectin gene were as follows: at position +45, 249 subjects (72.6%) had the common T/T genotype, 87 (25.4%) were T/G heterozygotes, and 7 (2.0%) were homozygous for the rare G allele (G allele frequency = 0.15). At position +276, 172 subjects (51.3%) were G/G, 136 (40.6%) were G/T, and 27 (8.1%) were T/T (T allele frequency = 0.28). Genotype distributions did not deviate from Hardy–Weinberg expectations for both +45T>G and +276G>T SNPs ($P=0.85$ and $P=0.99$ respectively). Strong pairwise LD was found between the two SNPs ($D'=-0.996$, $P<0.001$, $R^2=0.256$) with estimated +45/+276 haplotype frequencies of 0.57 for haplotype TG, 0.29 for TT, and 0.14 for GG.

Pearson correlation of plasma total, HMW adiponectin, and HOMA-IR with anthropometric and metabolic parameters

Plasma total and HMW adiponectin were highly correlated ($r=0.801$, $P<0.001$). The associations of plasma total adiponectin, HMW adiponectin, and

Table 2 Pearson correlation of plasma total adiponectin, HMW adiponectin, and homeostasis model assessment-insulin resistance (HOMA-IR) with anthropometric and metabolic parameters.

	Total adiponectin (n=349)		HMW adiponectin ^a (n=211)		HOMA-IR ^a (n=349)	
	r	P value	r	P value	r	P value
Age	0.194	<0.001	0.185	0.006	-0.053	0.32
Body mass index	-0.288	<0.001	-0.316	<0.001	0.517	<0.001
Body fat (kg)	-0.292	<0.001	-0.332	<0.001	0.514	<0.001
Body fat (%)	-0.215	<0.001	-0.254	<0.001	0.430	<0.001
Waist circumference	-0.304	<0.001	-0.333	<0.001	0.582	<0.001
Waist-to-hip ratio	-0.282	<0.001	-0.234	<0.001	0.416	<0.001
Triglycerides ^a	-0.231	<0.001	-0.267	<0.001	0.340	<0.001
Total cholesterol	0.132	0.01	0.060	0.374	0.012	0.82
LDL cholesterol	0.130	0.02	0.035	0.603	0.007	0.90
HDL cholesterol ^a	0.336	<0.001	0.367	<0.001	-0.263	<0.001
ApoA1	0.317	<0.001	0.262	<0.001	-0.172	0.001
ApoB	-0.073	0.182	-0.029	0.666	0.06	0.27
Fasting glucose	-0.259	<0.001	-0.142	0.04	—	—
Fasting insulin ^a	-0.272	<0.001	-0.384	<0.001	—	—
HOMA-IR ^a	-0.310	<0.001	-0.383	<0.001	—	—
HMW adiponectin ^a	0.801	<0.001	—	—	-0.383	<0.001

HMW adiponectin, high-molecular weight adiponectin. Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) with the following formula: HOMA-IR=fasting insulin (μU/ml)×fasting glucose (mmol/l)/22.5.

^aTested on log-transformed values.

HOMA-IR with anthropometric and metabolic parameters were all in the expected directions (Table 2). Total and HMW adiponectin concentrations were negatively correlated with BMI, BF, waist circumference, waist-to-hip ratio, fasting triglyceride, glucose, and insulin concentrations, as well as with HOMA-IR.

In addition, plasma total and HMW adiponectin were positively correlated with HDL cholesterol and apoA1 levels. HOMA-IR exhibited reverse correlation patterns with anthropometric parameters, plasma triglycerides and HDL cholesterol concentrations (Table 2).

Table 3 Association of adiponectin +45T>G and +276G>T variants with plasma and anthropometric measures.

	SNP +45T>G genotype (rs2241766)				SNP +276G>T genotype (rs1501299)			
	T/T (n=249)	T/G (n=87)	G/G (n=6)	P value*	G/G (n=171)	G/T (n=136)	T/T (n=27)	P value*
Age (years)	47.2±12.3	46.8±11.6	47.7±12.0	0.96	46.0±12.0	47.8±12.1	48.8±13.9	0.31
Body mass index (kg/m ²)	29.0±5.8	28.7±4.6	27.2±5.8	0.81	28.4±4.9	29.3±5.8	29.8±7.1	0.22
Body fat (%)	40.9±6.6	41.2±6.1	38.6±8.7	0.68	40.7±6.5	41.2±6.1	41.6±8.3	0.76
Waist circumference (cm)	86.0±12.6	85.5±9.5	80.3±9.7	0.63	85.2±11.1	86.3±12.4	87.1±14.4	0.62
Waist-to-hip ratio	0.78±0.06	0.79±0.05	0.74±0.03	0.18	0.79±0.06	0.79±0.06	0.78±0.07	0.56
Total adiponectin (μg/ml)	14.31±7.00	14.79±7.84	13.95±5.58	0.67	14.07±7.43	14.73±6.81	17.35±6.84	0.13
HMW adiponectin (μg/ml) ^{a,b}	5.59±3.77	6.37±4.36	5.07±3.03	0.17	5.95±4.17	5.42±3.41	6.30±4.63	0.37
Fasting insulin (μU/ml) ^a	8.89±4.76	7.99±4.24 [‡]	6.30±2.13	0.05	8.07±3.96	9.35±5.22 ^{‡,§}	9.65±5.91	0.01
Fasting glucose (mg/dl)	95.4±11.0	94.8±10.8	97.5±10.4	0.54	94.4±10.6	95.9±11.0	96.7±11.0	0.60
HOMA-IR ^a	2.12±1.23	1.89±1.01 [‡]	1.50±0.48	0.07	1.90±1.00	2.23±1.28 ^{‡,§}	2.38±1.63	0.009
Triglycerides (mg/dl) ^a	95.6±47.9	96.2±49.8	68.0±20.1	0.48	95.1±47.7	96.0±50.1	95.9±45.7	0.96
Total cholesterol (mg/dl)	218.8±43.5	220.4±45.9	208.7±24.1	0.86	220.0±44.8	217.4±42.7	221.3±42.9	0.60
LDL cholesterol (mg/dl)	147.7±35.7	149.1±37.0	141.2±18.1	0.92	148.7±36.6	146.5±34.8	150.8±35.0	0.65
HDL cholesterol (mg/dl) ^a	51.8±11.1	52.1±10.7	54.0±7.7	0.84	52.2±11.4	51.5±10.7	51.4±9.4	0.67
Total cholesterol/HDL	4.34±0.95	4.33±0.92	3.90±0.47	0.84	4.33±0.96	4.33±0.93	4.38±0.91	0.99
ApoA1 (mg/dl)	128.0±20.9	130.1±24.4	130.6±12.7	0.62	130.4±23.5	127.0±20.0	125.9±18.0	0.15
ApoB (mg/dl)	85.9±21.3	86.3±23.0	81.0±18.1	0.93	87.2±22.7	83.7±20.4	86.1±22.8	0.18

Values are unadjusted means ± s.d. *Overall P values derived by one-way analysis of covariance with a general linear model adjusting for age, estrogen use, and menopausal status for anthropometric parameters, plus smoking status, and % body fat for all other variables. [‡]P=0.01; [§]P=0.08 by Bonferroni *post-hoc* tests compared with subjects homozygous for the common allele. [§]P=0.004; ^{||}P=0.08 by least significance difference *post-hoc* tests compared with subjects homozygous for the common allele. Bold indicates significant results.

^aSignificance was tested on log-transformed values.

^bGenotype distribution for subjects with high-molecular weight (HMW) adiponectin measurements was as follows: T/T (n=156), T/G (n=52), G/G (n=3) for SNP +45T>G and G/G (n=99), G/T (n=91), T/T (n=21) for SNP +276G>T.

Effect of +45T>G and +276G>T SNPs on anthropometric and metabolic parameters

There were no significant genotype-related differences in anthropometric parameters, plasma concentrations of glucose, triglyceride, total, LDL, and HDL cholesterol or plasma concentrations of total and HMW adiponectin at positions +45 or +276 (Table 3). Furthermore, the ratio of HMW to total adiponectin was not different across genotypes for the two SNPs (data not shown). However, in multivariate analysis controlling for the potential confounders such as age, estrogen use, menopausal status, smoking status, and BF content (or BMI), both SNPs showed evidence of association with fasting insulin levels and insulin resistance (Table 3). Carriers of the rare +45G allele (T/G+G/G subjects) exhibited lower levels of fasting insulin compared with T/T homozygotes (7.88 ± 4.15 vs 8.89 ± 4.76 μ IU/ml, $P=0.02$) and had lower HOMA-IR index (1.86 ± 0.99 vs 2.12 ± 1.23 , $P=0.02$). SNP +276G>T was also significantly associated with fasting insulin and HOMA-IR. Compared with G/G homozygotes, carriers of the +276T allele (G/T+T/T) had higher fasting insulin levels and HOMA-IR index (9.40 ± 5.32 vs 8.07 ± 3.96 μ IU/ml, $P=0.002$, and 2.25 ± 1.34 vs 1.90 ± 1.00 , $P=0.002$ respectively). Further adjustment for alcohol consumption and physical activity level (data available for 290 women) resulted in similar findings (data not shown).

Effect of +45/+276 haplotypes on circulating adiponectin concentrations

Although single SNP analysis showed no significant associations with circulating adiponectin concentrations, a significant association with plasma total adiponectin was found at the haplotypic level ($P=0.03$; Fig. 1). We divided our sample into three haplotype groups according to the most common haplotype TG: i) TG/TG group (homozygous TG haplotype, i.e. individuals who were T/T at position +45 and G/G at position +276), ii) TG/X group (heterozygous TG haplotype, TG/TT+TG/GG), and iii) X/X group (all other haplotypes, GG/GG+GG/TT+TT/TT). Homozygous carriers of the TG haplotype exhibited lower plasma total adiponectin concentrations compared with noncarriers of this haplotype (X/X group; 13.71 ± 7.12 vs 16.40 ± 6.68 μ g/ml, Bonferroni P value=0.02, Fig. 1). A similar trend was observed for HMW adiponectin, albeit not statistically significant, whereas the ratio of HMW to total adiponectin was not different between the haplotype groups (data not shown). No significant haplotype effects on any other blood or anthropometric measures were observed (data not shown).

Figure 1 Total adiponectin concentrations according to carrying status of adiponectin +45T/+276G haplotype ($n=332$). Bars represent mean values $\pm$ s.e.m. P values adjusted for age, estrogen use, menopausal status, smoking status, and body fat. * $P=0.020$ by Bonferroni *post-hoc* tests, $P=0.007$ by least significant difference *post-hoc* tests compared with non-TG haplotype carriers (X/X group). ** $P=0.036$ by least significant difference *post-hoc* tests compared with group X/X.

Interaction between ADIPOQ gene SNPs and BF content on insulin resistance

In regression analysis and in ANCOVA, SNPs +45 and +276 did not show an independent role on HOMA-IR, with BF (expressed either as kg or percentage) being the strongest predictor of it ($P<0.001$), thus suggesting that the adiponectin gene may interact with BF in determining IR. The possible interaction between the adiponectin SNP genotypes and BF on IR was tested by subdividing the study group into two categories according to % BF (i.e. below or above the median value of 41% of the entire cohort). We found a significant interaction between BF (%) and genotype at position +45 for plasma insulin levels ($P=0.013$) and HOMA-IR ($P=0.017$), while an interaction of borderline significance between BF and genotype at position +276 was also observed for these IR parameters (P for both interactions=0.07). As shown in Fig. 2, the observed differences in HOMA-IR according to SNP +45 and +276 genotypes were very significant among women with a higher BF content ($\geq 41\%$), but not among leaner individuals. Specifically, only in the subgroup of women with $BF \geq 41\%$, a significant association of the +45G allele with lower insulin levels (GG+TG: 8.71 ± 5.22 versus TT: 10.70 ± 5.54 μ IU/ml, $P=0.003$) and HOMA-IR index (GG+TG: 2.11 ± 1.19 versus TT: 2.63 ± 1.42 , $P=0.005$) was observed, as well as a significant association of the +276T allele with higher insulin levels (TT+GT: 11.55 ± 6.50 versus GG: 9.23 ± 4.43 μ IU/ml, $P=0.002$) and HOMA-IR (GG+TG: 2.84 ± 1.60 versus TT: 2.24 ± 1.10 , $P=0.002$).

Figure 2 Effect of adiponectin gene +45 and +276 SNP status on HOMA-IR index according to percentage body fat categories (% BF < 41 versus % BF ≥ 41). Bars represent mean values ± S.E.M. *P* values adjusted for age, estrogen use, menopausal status, smoking status, and body fat.

In contrast, no difference in HOMA-IR between 'risk' genotype carriers and noncarriers was observed within subjects with BF < 41% (Fig. 2).

Discussion

In the present study, we assessed whether SNPs +45T>G (rs2241766) and +276G>T (rs1501299) in the *ADIPOQ* gene are associated with total and HMW adiponectin concentrations, metabolic parameters, measures of insulin sensitivity, and obesity in a healthy nondiabetic Greek women sample. Our findings provide evidence of an association between these two variants and IR. More precisely, a significant relationship between SNP +45 and IR was found, with +45G allele carriers exhibiting lower fasting insulin levels and HOMA-IR index than T/T subjects. An adverse effect of T allele at position +276 on IR was also observed, as carriers of this allele exhibited higher fasting insulin levels and had higher HOMA-IR. Interestingly, these effects were significant only in the population-specific group of women with higher BF (≥ 41%), thus suggesting that the *ADIPOQ* gene effects on insulin sensitivity are dependent upon BF status.

SNPs +45T>G and +276G>T have been studied in several populations including Europeans, Asians, and Americans (1, 33). Although there is a lack of consistency among studies, the results indicate that

genetic variation in the *ADIPOQ* gene is associated with IR and T2DM (8, 10–12, 20, 25, 28, 34, 35). Moreover, the strong LD with T2DM, insulin levels, and metabolic syndrome, found in the chromosomal region where the *ADIPOQ* gene is located (36, 37), suggests that somewhere in this locus common genetic variant(s) may have a measurable effect on IR-related phenotypes. In accordance with the findings of the present study, Yang *et al.* (37) first reported lower insulin sensitivity for +45T allele in a Chinese and Japanese population, while in a Mediterranean population of Italy +276T allele was associated with higher insulin levels and HOMA-IR (24). However, other studies have provided opposite results (8, 11, 20, 28). In nondiabetic Germans, the +45G allele was associated with higher IR indexes (11), whereas in nondiabetic Korean, as well as in Japanese men, +276T allele was found to be protective for IR (association with lower HOMA-IR) (8, 28). These conflicting association results in various populations suggest a complex relationship between *ADIPOQ* gene variation and IR.

Because of the fact that SNP +45T>G is a synonymous mutation (GGT→GGG, Gly15Gly) at exon 2 and SNP +276G>T locates at intron 2, the exact molecular mechanisms responsible for the biological effects on IR cannot be elucidated so far. Yang *et al.* (19) showed that the silent +45T>G mutation may alter RNA splicing or stability, suggesting an allele-specific differential expression of adiponectin. This finding supports the fact that SNPs with no apparent biological significance may have an effect on gene expression, although it is very plausible for SNP +45 to be in LD with some other functional genetic alterations resulting in the difference in mRNA expression of its two alleles. SNP +276G>T could influence insulin sensitivity as it has been reported that intronic SNPs may modulate gene expression levels (38), although the influence on IR may simply reflect the LD between this SNP and a functional SNP for which it acts as a marker.

We could also speculate that different LD patterns among ethnic groups might be responsible for the variation of *ADIPOQ* SNPs in modulating IR, but conflicting results have been reported even between samples from the same ethnicity (10, 25). Another possible explanation could be the interaction between genotype and environmental factors such as diet and physical activity level. Different metabolic environments, including obesity and diabetic or prediabetic state, could affect the regulation of the *ADIPOQ* gene and the influence of SNPs within it. With regard to this notion, we found that the impact of *ADIPOQ* +45 and +276 variations on HOMA-IR index is dependent on the degree of obesity. It is possible that the contribution of these SNPs on IR is too small to be detected in lean subjects, but significant in more obese states where the risk of diabetes is greater. Our findings are in accordance with previously published data by Jang *et al.* (27) where

the phenotypic expression of the 'risk' genotype for SNP +276G>T was observed only among subjects with elevated BMI. Moreover, in nondiabetic Korean women, the association of the +45T/+276G haplotype with IR parameters was significant only among the overweight/obese subgroup (17). However, in an Italian nondiabetic population, the greater IR observed among subjects with the at-risk TT genotype was more pronounced in lean compared with obese individuals of this study (25).

In the current study, a reasonable implication is that the genetic effect of SNPs +45 and +276 on IR is mediated through an alteration of gene expression that would eventually affect circulating adiponectin levels. Nevertheless, single SNP analysis did not reveal any significant association of SNPs +45 and +276 with plasma total adiponectin levels. This discrepancy has also been shown in other studies where both SNPs did relate to IR but not to total adiponectin concentrations (8, 28). A possible explanation is that current adiponectin assays fail to differentiate between types of circulating adiponectin, as not all the circulating forms may be functional (1); however, in our study, both total and HMW adiponectin, as well as the ratio of HMW to total adiponectin, were not different across the two SNP genotype groups. In addition, two genomic scans in northern European (39) and Pima Indian (40) populations have shown that the *ADIPOQ* gene has only a small effect on plasma adiponectin levels. Considering that the *ADIPOQ* variation at positions +45 and +276 does not affect plasma adiponectin levels, we cannot rule out the hypothesis that there might be an influence on adiponectin concentration at the hormone's action sites, such as target tissues and mainly adipose tissue, through a local autocrine and/or paracrine effect. It is interesting that, while each SNP alone did not present any effect on plasma adiponectin concentrations in the SNP +45/+276 haplotype test, we found that homozygous carriers of the common TG haplotype had significantly lower concentration of plasma total adiponectin compared with noncarriers of this haplotype, although the 'risk' haplotype did not associate with other components of IR. Recently published data also support a lowering effect of TG haplotype on circulating levels of adiponectin (27, 41).

In conclusion, prior data on adiponectin SNPs and IR have been inconsistent. Data of the present study suggest a significant role of *ADIPOQ* variants at positions +45 and +276 in the development of IR in healthy Greek women possibly through an interaction with BF. Further studies are needed to directly assess the influence of these two common variants on *ADIPOQ* gene expression and the potential mechanism by which they modulate susceptibility to IR.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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