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Adiponectin and adiponectin receptor gene variants in relation to resting metabolic rate, respiratory quotient, and adiposity-related phenotypes in the Québec Family Study[1–3]

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ABSTRACT

Background: Despite adiponectin's presumed role in fatty acid oxidation and energy homeostasis, little is known about the effect of gene variants on substrate oxidation, energy expenditure, and adiposity-related phenotypes.

Objective: We examined the effects of genetic variation in adiponectin (*ADIPOQ*) and adiponectin receptors 1 and 2 (*ADIPOR1* and *ADIPOR2*) on resting metabolic rate, respiratory quotient (RQ), and adiposity-related phenotypes.

Design: We studied the associations of *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* polymorphisms with resting metabolic rate, RQ, and body mass index, percentage body fat, sum of 6 skinfold thicknesses, waist circumference, and total, subcutaneous, and visceral fat in 759 participants in the Québec Family Study.

Results: The *ADIPOQ* 45T→G single-nucleotide polymorphism (SNP) was significantly ($P = 0.0002$ to 0.04) associated with overall adiposity and abdominal adiposity; the rare homozygotes (*G/G*) had a leaner phenotype than did the carriers of the common allele. One SNP each in the putative promoter of *ADIPOR1* (ie, −3882T→C) and *ADIPOR2* (ie, IVS1 −1352G→A) was associated with RQ ($P = 0.03$ and 0.04, respectively), and the association was even stronger in nonobese persons ($P = 0.02$ and 0.003). Carriers of the common alleles (*ADIPOR1 T* and *ADIPOR2 G* alleles) had a lower RQ than did the rare homozygotes. A significant genotype-by-genotype interaction ($P = 0.0002$ to 0.02) was found between SNPs in the promoters of *ADIPOQ* (−3971A→G) and *ADIPOR1* (−3882T→C). Subjects carrying the minor *ADIPOQ* allele (*G* allele) who were rare homozygotes (*C/C*) for the *ADIPOR1* SNP had a higher RQ ($P = 0.003$) and greater overall ($P < 0.03$) and abdominal ($P < 0.05$) adiposity than did persons with other genotype combinations.

Conclusions: Previous findings that the *ADIPOQ* 45T→G variant contributes to overall fatness and abdominal obesity are confirmed. Moreover, variants in the promoter region of both *ADIPOR* genes contribute to substrate oxidation. *Am J Clin Nutr* 2007;85:26–34.

KEY WORDS Adiponectin, adiponectin receptor, resting metabolic rate, respiratory quotient, obesity, abdominal obesity, adiposity

INTRODUCTION

Adiponectin is a protein that is produced exclusively and abundantly in the adipose tissue and that is present in plasma in relatively high concentrations (1). Unlike the concentrations of most other adipokines, such as tumor necrosis factor α, interleukin 6, leptin, and resistin, those of adiponectin are low in patients with obesity and type 2 diabetes (2, 3) and are inversely associated with measures of overall adiposity (2–5) and with insulin resistance, independent of fat mass (5). The administration of adiponectin in animal models of obesity and diabetes results in weight loss, prevention of diet-induced obesity without influencing food intake, reduced tissue triacylglycerol content in muscle and liver, increased fatty acid oxidation in skeletal muscle, and improved insulin sensitivity and glucose tolerance (4, 6, 7). Adiponectin is encoded by the adiponectin gene, *ADIPOQ*, located on chromosome 3q27 in humans, which coincides with a diabetes susceptibility locus (8). In humans, adiponectin and *ADIPOQ* gene polymorphisms have been studied extensively for their effects on obesity- and diabetes-related phenotypes (2–5). Despite the presumed role of adiponectin in fatty acid oxidation and energy homeostasis, only 2 studies in humans have evaluated

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TABLE 1
Descriptive characteristics of participants[1]

	Men		Women		
	Parent (n = 166)	Offspring (n = 172)	Parent (n = 202)	Offspring (n = 219)	P
Age (y)	54.0 ± 7.5	28.4 ± 8.7	51.7 ± 7.6	29.5 ± 9.8	<0.001[2]
Weight (kg)	83.3 ± 18.4	80.0 ± 20.4	70.8 ± 19.8	70.5 ± 21.8	<0.001[3]
BMI (kg/m^2)	28.5 ± 6.1	26.1 ± 6.4	28.2 ± 7.7	27.0 ± 8.6	<0.001[2]
Percentage body fat (%)	26.5 ± 7.0	19.8 ± 9.5	35.1 ± 8.6	29.7 ± 10.5	<0.001[2,3]
ST6 (mm)	94 ± 41	78 ± 45	141 ± 61	120 ± 63.6	<0.05[2], <0.001[3]
TER	2.41 ± 0.57	2.01 ± 0.52	1.28 ± 0.32	1.19 ± 0.29	<0.001[2,3]
Waist circumference (cm)	98.6 ± 14.6	88.5 ± 16.9	86.0 ± 17.1	80.9 ± 17.8	<0.001[2,3]
ASF (cm^2)	202 ± 119	154 ± 120	307 ± 150	267 ± 169	<0.05[2], <0.001[3]
AVF (cm^2)	146 ± 77	75 ± 48	109 ± 58	61 ± 39	<0.001[2,3]
ATF (cm^2)	355 ± 184	234 ± 165	422 ± 198	332 ± 204	<0.001[2,3]
RMR (kcal/d)[4]	1650 ± 283	1742 ± 280	1323 ± 236	1412 ± 245	<0.01[2], <0.001[3]
RQ at rest	0.805 ± 0.048	0.793 ± 0.051	0.793 ± 0.049	0.786 ± 0.053	<0.01[2]

[1] All values are $\bar{x}$ ± SD. ST6, sum of the 6 skinfold thicknesses; TER, the trunk-to-extremity ratio; ASF, abdominal subcutaneous fat; AVF, abdominal visceral fat; ATF, abdominal total fat; RMR, resting metabolic rate; RQ, respiratory quotient. Student's t test was used to compare men to women and parents to offspring. No significant sex × generation interactions were found.
[2] Difference by generation.
[3] Difference by sex.
[4] 1 kcal = 4.186 kJ.

adiponectin concentrations in relation to substrate oxidation and energy expenditure (EE) (9, 10). In a Finnish study that included 158 offspring of persons with type 2 diabetes, adiponectin concentrations during hyperinsulinemia were related positively to EE and negatively to lipid oxidation rates (9). However, in 75 nondiabetic Pima Indians and 18 nondiabetic whites, no such associations were found with plasma adiponectin concentrations (10).

Much less is known about the recently discovered adiponectin receptors, encoded by the *ADIPOR1* and *ADIPOR2* genes, that are located at 1p36.13-q41 and 12p13.31, respectively (11). Both receptors are expressed in human skeletal muscle; *ADIPOR1* expression is more abundant than *ADIPOR2* expression (11, 12). In vitro experiments, in which cultured myocytes were treated with adiponectin, showed that suppression of the expression of the *ADIPOR* gene greatly reduced fatty acid oxidation and glucose uptake, whereas overexpression of *ADIPOR1* increased fatty acid oxidation and glucose uptake beyond the effects of adiponectin (11). *ADIPOR* gene expression was significantly lower in those Pima Indian subjects with a strong family history of type 2 diabetes than in those with no family history (12). However, the association between *ADIPOR* expression and insulin sensitivity could not be confirmed by other investigators (13). Common gene variants in the *ADIPOR* genes have been studied for associations with diabetes-related phenotypes (14–17). However, to the best of our knowledge, no studies have reported on the effect of common *ADIPOR1* and *ADIPOR2* gene variants on EE or substrate oxidation rates, despite their known roles in lipid oxidation and glucose uptake.

In the current study, we examined whether sequence variations in the *ADIPOQ* and *ADIPOR* genes contributed to variation in adiposity, EE, and fasting respiratory quotient (RQ) in participants in the Québec Family Study (QFS).

SUBJECTS AND METHODS

Study population

The aims and design of the QFS cohort have been described previously (18). Briefly, the QFS aimed to study the genetics of obesity and its related phenotypes by applying the most recent advances in genetic epidemiology available: ie, segregation analyses, linkage analyses, and association analyses. The QFS was initiated in 1978 and has consisted of 3 phases; phase 1 was based on 375 families, of which a subsample of 105 families and an additional 74 families were followed 10 y later in phase 2. Most of these families were remeasured 5 y later in phase 3. In the current study, which is based on phase 2 of the QFS, 759 subjects from 183 French-Canadian families living in the Greater Québec City area were included (**Table 1**).

Written informed consent was obtained from all participants. The QFS project was approved by the Medical Ethics Committee of Laval University.

Phenotypes

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, by using a stadiometer and a balance-beam scale. Body mass index (BMI; in kg/m^2) was calculated. Body density, obtained from underwater weighing (19), was converted to percentage body fat (%BF) by using the equation of Siri (20). Waist circumference was measured to the nearest 0.1 cm at the level of noticeable waist narrowing by using an anthropometric fiberglass tape in accordance to the procedures recommended by Lohman et al (21). Six measures of skinfold thicknesses (ie, suprailiac, subscapular, abdominal, medial calf, biceps, and triceps) were obtained on the left side of the body by using a Harpenden skinfold caliper and following the procedures recommended by the International Biological Program (22). We calculated the sum of the 6 skinfold thicknesses (ST6) as an

indicator of subcutaneous fatness and the ratio of trunk to extremity [(trunk:extremity) ie, (suprailiac + subscapular + abdominal):(medial calf + biceps + triceps)] as an indicator of preferential deposition of subcutaneous fat on the trunk rather than on extremities. Abdominal fat (total, visceral, and subcutaneous) was measured by using computed tomography scans (Somatom DRH scanner; Siemens, Erlanger, Germany) as described previously (23). Briefly, an abdominal scan was taken between the 4th and 5th lumbar vertebrae while participants were in a supine position with the arms stretched above the head. A radiograph of the skeleton was used as reference to determine the position of the scan to the nearest millimeter. Total and visceral adipose tissue areas were delineated with a graph pen. The attenuation interval used in the quantification of the areas of adipose tissue was between −190 and −30 Hounsfield units. The abdominal visceral fat (AVF) area was defined by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous fat (ASF) area was calculated by subtracting the AVF area from the abdominal total fat (ATF) area.

Resting metabolic rate (RMR) and RQ at rest were measured simultaneously with the use of a ventilated hood coupled to an open-circuit indirect calorimeter, as described previously (24). Measurements were made early in the morning, after an overnight fast, while participants were resting quietly in a semireclined position. Respiratory exchange data from the final 10 min of the 30-min data collection period were used to calculate RMR and RQ. Gas samples were assayed with a zirconia cell oxygen analyzer (Amatek CD-3A; Thermox Instruments Division, Pittsburgh, PA) and an infrared carbon dioxide analyzer (Amatek S-3A; Thermox Instruments Division). Analyzers were calibrated before each test with the use of gases of known percentages of oxygen and carbon dioxide. RMR is expressed in kcal/min EE, whereas RQ is simply the ratio of carbon dioxide produced to oxygen consumed.

Genotypes

Genomic DNA was prepared from permanent lymphoblastoid cells by using proteinase K and the phenol-chloroform technique. DNA was dialyzed 4 times against buffer [10 mmol Tris/L, 1 mmol EDTA/L; pH 8.0 (TE buffer)] for 6 h at 4 °C and precipitated.

We typed 3 SNPs in the *ADIPOQ* gene (**Figure 1**A)—IVS2 276G→T (rs1501299), exon 2 (EX2) 45T→G (rs2241766) and IVS1 −3971A→G (rs822396). The IVS2 276G→T and EX2 45T→G SNPs are the most extensively studied ADIPOQ SNPs and are located in the second of 2 linkage disequilibrium (LD) blocks, whereas the IVS1 −3971A→G SNP is located in the first LD block (25). The IVS2 276G→T polymorphism was detected with the *BsaM*I restriction enzyme (call rate: 98.0%), and the EX2 45T→G polymorphism with the *Sma*I restriction enzyme (call rate: 99.8%). The genomic DNA (20 ng) was amplified in 6-μL mixtures containing dNTPs (30 μmol/L each), Taq DNA polymerase (0.3 U), buffer (15 mmol $MgCl_2$/L), and polymerase chain reaction (PCR) primers (50 nmol/L each). The reaction mixture was denaturated for 5 min at 95 °C, followed by 34–38 PCR amplification cycles. The first 10 cycles consisted of denaturation at 95 °C for 20 s, primer annealing at 57 °C for 1 min, and primer extension at 72 °C for 15 s. The following 24–28 cycles consisted of denaturation at 94 °C for 20 s, primer annealing at 52 °C for 55 s, and primer extension at 72 °C for 15 s. The PCR mixture was then digested by the restriction enzyme for 15 min at 37 °C and then for 20 min at 80 °C. A mini-sequencing assay was performed according to Sun et al (26): dATP/ddNTP mix (1.56 μmol/L each), IRDye tag primers (3.125 nmol/L), Thermosequenase (0.3 U), and 0.6X buffer were added to the PCR mixtures. Subsequently, the mixture was denaturated for 2 min at 95 °C and then subjected to 30 amplification cycles. Each cycle consisted of denaturation at 96 °C for 10 s, primer annealing at 57 °C for 30 s, and primer extension at 72 °C for 15 s.

The *ADIPOQ* IVS1 −3971A→G SNP and the SNPs in the *ADIPOR1* (ie, rs1539355 and rs2275737) and *ADIPOR2* (ie, rs10773982 and rs2058112) genes were typed by using the Acycloprime Fluorescent Polarization SNP detection kit (Perkin Elmer Life Sciences, Inc, Boston, MA), which includes PCR amplification, amplicon purification, and dye-terminator incorporation reaction (call rates for the SNPs: 97.7%, 92.2%, 97.0%, 95.0%, and 98.0%, respectively). The genomic DNA (25 ng) was amplified in 6-μL reaction mixtures containing 0.5 μL 10× PCR buffer (Applied Biosystems), 0.4 μL 25 mmol $MgCl_2$/L (2), 0.1 μL 5 mmol dNTP/L, 0.06 μL PCR primers (10 μmol/L each), 0.02 μL Taq polymerase, and 4.86 μL H_2O. The reaction mixture was held at 95 °C for 2 min for Taq enzyme activation and then underwent 35 amplification cycles. Each cycle consisted of denaturation at 95 °C for 10 s, primer annealing at 60 °C for 20 s, and primer extension at 68 °C for 30 s. The reaction mixtures were then incubated at 68 °C for 10 min for final primer extension. Unused primers and unincorporated dNTPs were removed by using clean-up reagent (0.2 μL), clean-up dilution buffer (1.8 μL; Perkin Elmer Life Sciences), and H_2O (3 μL) and then incubated at 37 °C for 60 min and at 80 °C for 15 min for enzyme inactivation.

For the primer extension reaction, the purified PCR product was combined with 0.05 μL acyclopol, 2.0 μL 10× reaction buffer, 0.5 μL of the specific dye terminator combination (Perkin Elmer Life Sciences), 0.5 μL SNP primer (10 μmol/L), and 6.95 μL H_2O. The reaction mixtures were then incubated at 95 °C for 2 min and the underwent 5–15 cycles, each at 95 °C for 15 s and at 55 °C for 30 s. Fluorescent polarization values were directly measured by using a plate reader (VICTOR^2; Perkin Elmer Life Sciences). The 4 SNPs in the *ADIPOR* gene were selected on the basis of information that was available from the Centre d'Etude du Polymorphisme Humain population (specifically, Utah residents with Northern and Western European ancestry) of the International HapMap Project (27). We selected the SNPs in such way that the linkage disequilibrium between them is low; ie, each SNP independently represents a part of the genetic variation of the gene. Therefore, we used the SNPSpD (Single Nucleotide Polymorphism Spectral Decomposition; Internet: http://genepi.gimr.edu.au/general/daleN/SNPSpD) Web interface that, on the basis of spectral decomposition of matrixes of pairwise LD between SNPs, enables the selection of a subset of SNPs that optimize the information in a genomic region (28).

Statistical analysis

Associations between the *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* SNPs and the phenotypes were analyzed by using the MIXED model procedure in SAS software (version 8.2**;** SAS Institute Inc, Cary, NC). Nonindependence among family members was adjusted for by using a "sandwich estimator" that asymptotically yields the same parameter estimates as ordinary least-squares or regression methods, but the standard errors and resulting hypothesis tests are adjusted for the dependencies. The

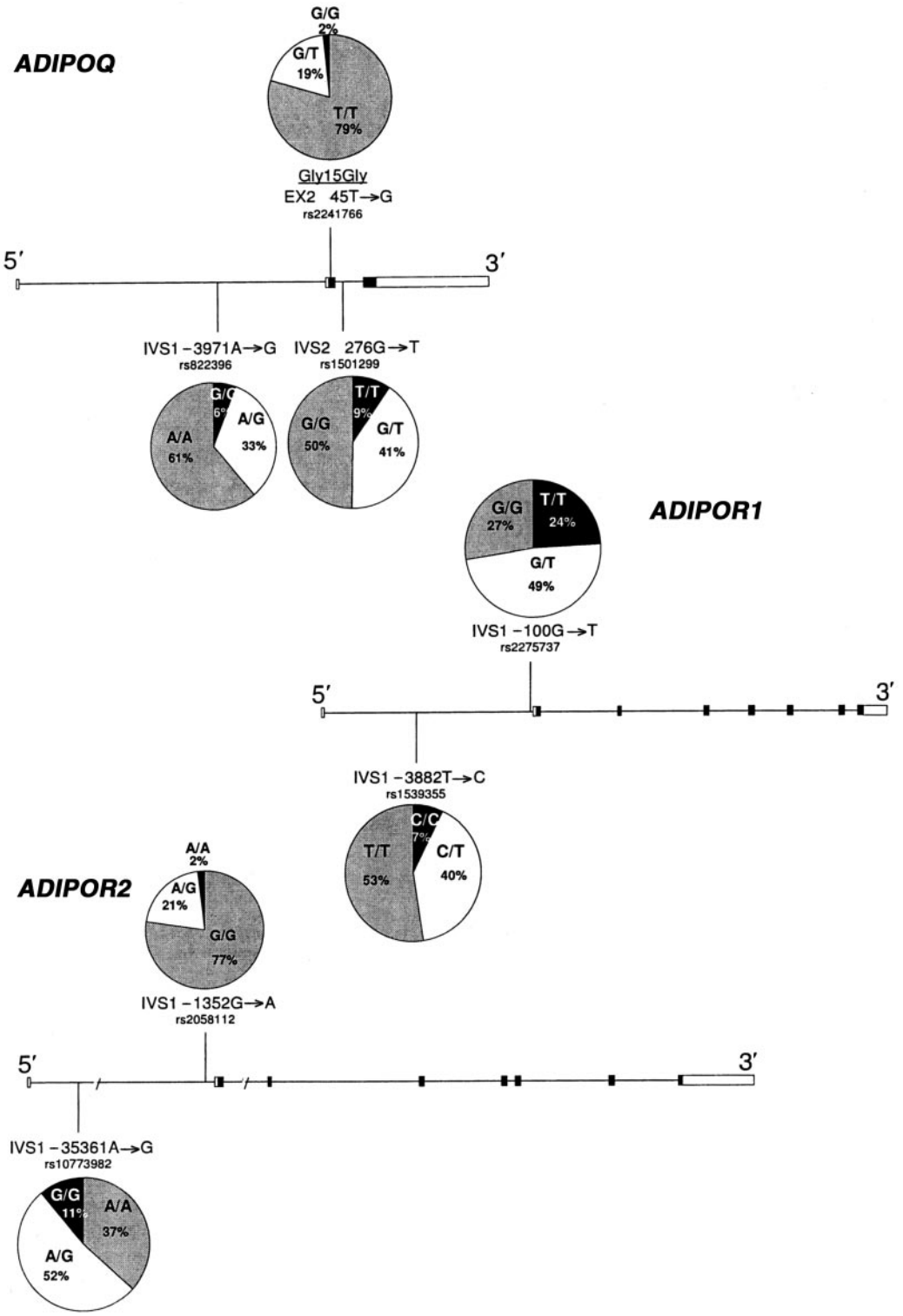

FIGURE 1. Location and genotype frequencies of the single-nucleotide polymorphisms in the adiponectin (*ADIPOQ*) and adiponectin receptor 1 and 2 (*ADIPOR1* and *ADIPOR2*, respectively) genes, according to parental data.

method is general and assumes the same degree of dependency among all members within a family. The general-linear-model procedure was used to analyze associations between the parents, because they are not genetically related. Possible gene-by-gene, sex-by-gene. and BMI (<30 or ≥ 30)-by-gene interactions were tested with the MIXED model procedure by including main effects and interaction terms in the same model. Haplotypes were estimated by using the best option that is available in the MERLIN software (version 1.0.1; 29). Associations between haplotypes and phenotypes were performed by using the MIXED model procedure. Only subjects with unambiguous haplotypes were included in the analyses.

TABLE 2
Associations between the *ADIPOQ* EX2 45T→G polymorphism and the anthropometric measures resting metabolic rate (RMR) and respiratory quotient (RQ)[1]

	ADIPOQ EX2 45T→G						
	T/T		*T/G*		*G/G*		
	Value	Subjects	Value	Subjects	Value	Subjects	P^2
		n		*n*		*n*	
BMI (kg/m^2)	27.2 ± 0.3[3]	590	28.5 ± 0.6	147	25.8 ± 2.1	12	0.13
Percentage body fat (%)	27.2 ± 0.4	515	28.9 ± 0.8	129	20.7 ± 3.1	8	0.006
ST6 (mm)	104 ± 2.1	583	115 ± 4.7	146	75 ± 11.4	11	0.0002
TER	1.66 ± 0.02	583	1.59 ± 0.03	146	1.46 ± 0.11	11	0.08
Waist circumference (cm)	87.7 ± 0.7	587	91.2 ± 1.4	147	86.0 ± 4.8	12	0.09
ASF (cm^2)	222 ± 7	443	248 ± 15	100	146 ± 31	9	0.02
AVF (cm^2)	90 ± 2	443	101 ± 5	100	62 ± 11	9	0.10
ATF (cm^2)	323 ± 9	443	360 ± 20	100	220 ± 43	9	0.04
RMR (kcal/d)	1533 ± 7	550	1514 ± 13	138	1512 ± 53	11	0.98
RQ at rest	0.795 ± 0.002	550	0.793 ± 0.004	138	0.815 ± 0.017	11	0.63

[1] ADIPOQ, adiponectin; EX2, exon 2; ST6, sum of the 6 skinfold thicknesses; TER, the trunk-to-extremity ratio; ASF, abdominal subcutaneous fat; AVF, abdominal visceral fat; ATF, abdominal total fat.
[2] ANOVA with mixed model was used to compare the 3 genotypes.
[3] $\bar{x}$ ± SE (all such values).

RMR and RQ measurements were adjusted for sex, age, fat mass, and fat-free mass. ST6 and trunk:extremity were adjusted for sex, age, and height. Waist circumference, ASF, AVF and ATF were adjusted for sex and age. We log transformed ST6, trunk:extremity, ASF, AVF, and ATF variables before analyses to normalize their skewed distributions. The inverse log-transformed values are given in the tables.

A likelihood ratio test was performed to assess whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. The pairwise LD among the SNPs was assessed by r^2 and D' (normalized measure of Lewontin) by using the ldmax program as implemented in the GOLD software (8 November 2001 version; 30).

RESULTS

The genotype frequencies for the *ADIPOQ*, *ADIPOR1*, and *ADIPOR2* polymorphisms are shown in Figure 1. The naming of the SNPs is based on their intronic or exonic nucleotide change, and it reflects their relative position to the start codon. All genotype frequencies were in Hardy-Weinberg equilibrium. The pairwise LD among the *ADIPOQ* SNPs was generally low; ie, r^2 = 0.02 and D' = 0.71 between IVS1 −3971A→G and EX2 45T→G, $r^2 < 0.01$ and D' = 0.10 between IVS1 −3971A→G and IVS2 276G→T, and r^2 = 0.09 and D' = 0.86 between EX2 45T→G and IVS2 276G→T. For *ADIPOR1* SNPs, the pairwise LD between IVS1 −3882T→C and IVS1 −100G→T was r^2 = 0.34 and D' = 0.97; for ADIPOR2 SNPs, the pairwise LD between IVS1 −35361A→G and IVS1 −1352G→A was r^2 = 0.09 and D' = 0.99. The phenotypic characteristics of the 759 participants are shown in Table 1.

The *G/G* homozygotes of the EX2 45T→G *ADIPOQ* gene were significantly leaner and had less abdominal fat than did the carriers of the *T* allele. On average, the *G/G* homozygotes had %BF 6 percentage points lower, ST6 28 mm smaller, and ASF and ATF measures 75 cm^2 and 103 cm^2 lower, respectively, than did the *T/T* homozygotes (**Table 2**). None of the *ADIPOQ* variants had an association with RMR or RQ, and no associations were observed with the *ADIPOQ* IVS2 276G→T variant. Moreover, the *ADIPOQ* IVS1 −3971A→G variant was not directly associated with any of the studied phenotypes. However, significant genotype-by-genotype interactions (P = 0.0002 to 0.02) were observed between the *ADIPOQ* IVS1 −3971A→G variant and the *ADIPOR1* IVS1 −3882T→C variant (**Figure 2**). Carriers of the *ADIPOQ* IVS1 −3971A→G *G* allele who also were *C/C* homozygotes for the *ADIPOR1* −3882T→C variant had a significantly higher RQ (>0.03), higher %BF (>3.4%), and greater ST6 (>21 mm) and waist circumference (>4.8 cm) than did subjects with the other genotype combinations.

In each of the 2 *ADIPOR* genes, a 5'-untranslated region (UTR) variant had a significant association with RQ (**Table 3**). The rare homozygotes (*C/C*) of the *ADIPOR1* IVS1 −3882T>C variant had a significantly higher RQ than did the carriers of the *T* allele, and this difference was more pronounced in the nonobese subjects. Moreover, for the *ADIPOR2* IVS1 −1352G>A variant, the rare homozygotes (A/A) had a significantly higher RQ than did the *G* allele carriers. The association was significantly stronger in nonobese than in obese subjects (gene-by-obesity status interaction, P = 0.001). None of the *ADIPOR* gene variants had a direct association with any of the adiposity measures. The haplotypes did not provide any more information than did the individual markers.

DISCUSSION

SNPs in the *ADIPOQ* gene have frequently been associated with obesity-related phenotypes (31–39). However, not all studies support this hypothesis (40–44). Some studies have found *ADIPOR* gene DNA sequence variants to be related to type 2 diabetes and insulin resistance (17). However, to the best of our knowledge, no studies have reported associations between *ADIPOR* SNPs and fat oxidation rate, EE, and other adiposity-related phenotypes.

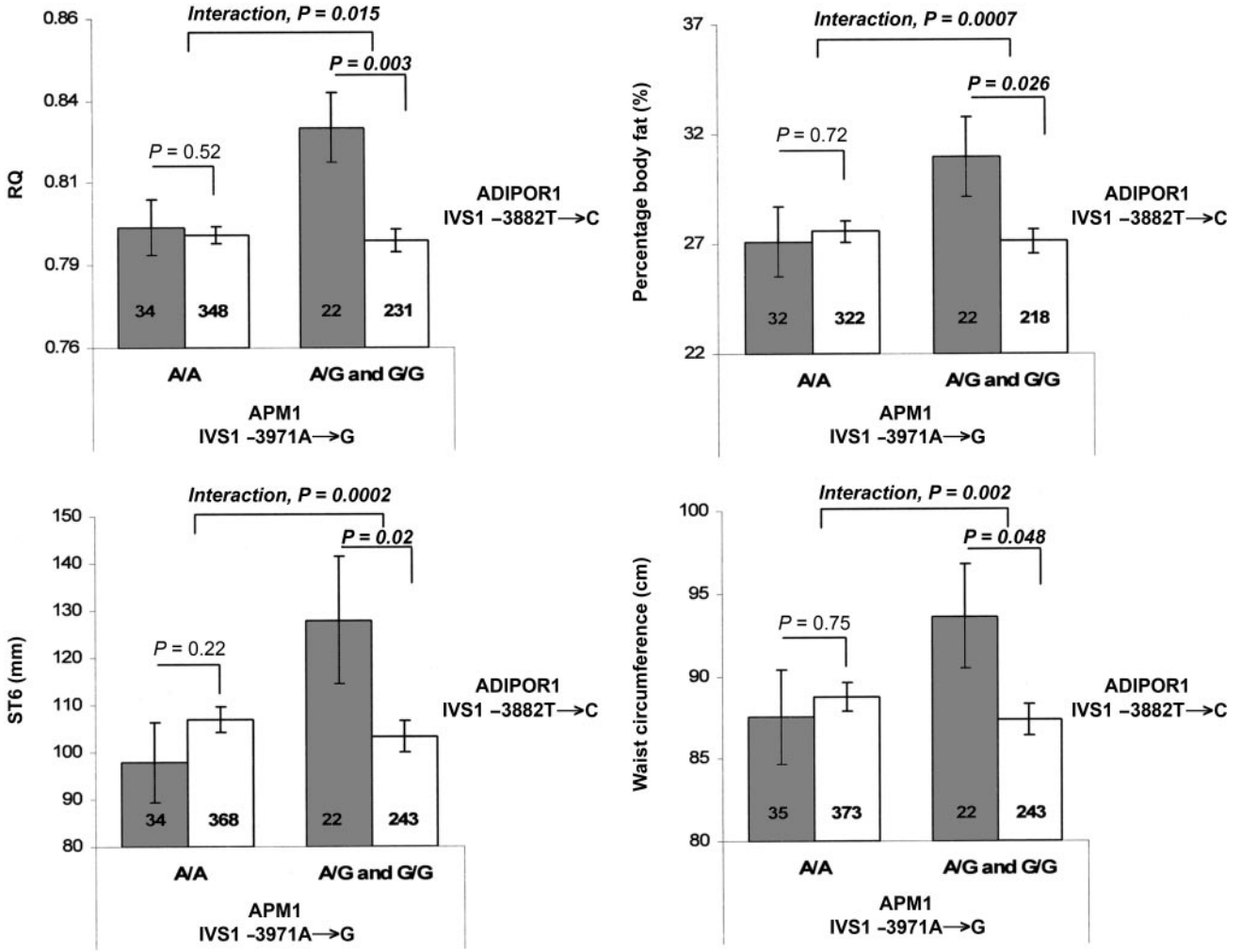

FIGURE 2. Genotype-by-genotype interaction: association between the IVS1 −3971A→G adiponectin (*ADIPOQ*) polymorphism and respiratory quotient (RQ), percentage body fat, sum of 6 skinfold thicknesses (ST6), and waist circumference in interaction with the IVS1 −3882T→C *ADIPOQ* receptor 1 (*ADIPOR1*) polymorphism. *APM1*, adiponectin gene 1. ▨, *C/C*; □, *C/T* and *T/T*. Genotype-by-genotype interactions were tested with the MIXED model procedure by including main effects and interaction terms in the same model.

Our data show that the *ADIPOQ* 45T→G variant is significantly associated with measures of overall adiposity and abdominal fatness and that the minor allele (*G/G*) homozygotes have a leaner phenotype than do the carriers of the common alleles (*T/T* and *G/T*). In addition, we found that one SNP each in the promoter region of *ADIPOR1* (IVS1 −3882T→C) and *ADIPOR2* (IVS1 −1352G→A), respectively, was associated with RQ, and this association was even stronger in nonobese persons. Carriers

TABLE 3
Associations between the *ADIPOR1* IVS1 −3882T→C and *ADIPOR2* IVS1 −1352G→A polymorphisms and respiratory quotient (RQ) in nonobese and obese subjects[1]

	ADIPOR1 IVS1 −3882T→C							*ADIPOR2* IVS1 −1352G→A						
	T/T		*T/C*		*C/C*			*G/G*		*A/G*		*A/A*		
	Value	Subjects	Value	Subjects	Value	Subjects	P^2	Value	Subjects	Value	Subjects	Value	Subjects	P^2
		n		*n*		*n*			*n*		*n*		*n*	
RQ at rest	0.792 ± 0.003	318	0.795 ± 0.003	272	0.809 ± 0.007	56	0.029	0.795 ± 0.002	520	0.794 ± 0.004	153	0.822 ± 0.013	16	0.041
Non obese	0.799 ± 0.003	241	0.803 ± 0.004	206	0.816 ± 0.008	38	0.018	0.801 ± 0.003	390	0.801 ± 0.005	115	0.837 ± 0.014	15	0.003
Obese	0.772 ± 0.005	77	0.769 ± 0.006	66	0.792 ± 0.011	18	0.15	0.773 ± 0.004	130	0.774 ± 0.008	38	0.728	1	0.85

[1] ADIPOR, adiponectin receptor. Genotype-by-obesity interaction was tested with the MIXED model procedure by including main effects and interaction terms in the same model. Gene-by-obesity status interaction; *ADIPOR1* IVS1 −*3882T→C*, $P = 0.07$ and *ADIPOR2* IVS1 −*1352G→A*, $P = 0.001$.
[2] ANOVA with mixed model was used to compare the 3 genotypes.

of the common alleles (*T* allele for *ADIPOR1* and *G* allele for *ADIPOR2*) had a higher fat oxidation rate (ie, lower RQ) than did the rare homozygotes (*C/C* and *A/A*, respectively). A significant genotype-by-genotype interaction was observed between SNPs in the proximal promoters of *ADIPOQ* (−3971A→G) and *ADIPOR1*, respectively (−3882T→C). Subjects carrying the rare *ADIPOQ* allele (*G* allele) who also were rare homozygotes (*C/C*) for the *ADIPOR1* variant had a lower fat oxidation rate (higher RQ) and higher measures of overall and abdominal adiposity than did subjects with the other genotype combinations.

The *ADIPOQ* EX2 45T→G, one of the most extensively studied *ADIPOQ* gene polymorphisms, is a synonymous mutation located in EX2. Because this SNP does not result in an amino acid change, it is unlikely that it has functional implications for protein activity. However, this SNP can be in complete or nearly complete LD with a yet unidentified polymorphism that has functional relevance. We found that the rare homozygotes (*G/G*) had significantly less overall body fat (%BF and ST6) as well as less abdominal fat (ASF and ATF) than did carriers of the common allele. Our findings have been supported by others (32, 34, 35, 37, 38). In the Swedish Obese Subjects cohort, rare homozygotes had a significantly smaller waist circumference than did the carriers of the common allele (34). Moreover, in a study of 713 Hispanic men and women, *G/G* homozygotes had a leaner phenotype—ie, lower BMI and smaller waist circumference and ASF—than did carriers of the *T* allele (37). Two other studies, one in nondiabetic whites (32) and the other in African Americans (35), found the *G* allele to be related to reduced measures of overweight and abdominal adiposity, but only when the EX2 45T→G SNP was part of a haplotype with IVS2 276G→T. These findings suggest that the *ADIPOQ* EX2 45T→G variant plays a protective role against the development of obesity. Several recent studies found the *G* allele to be associated with higher plasma adiponectin concentrations than was the *T* allele (45–47). This corroborates the findings in the current study, because higher adiponectin concentrations are correlated with lower body mass, BMI, and other obesity-related phenotypes (2–5, 10, 40). Other investigators, however, did not find an association between the EX2 45T→G variant and obesity-related phenotypes, which may be due to the fact that they examined the dominant model (*G/G* + *G/T* compared with *T/T*) because of small sample sizes (32, 35, 36, 40–42, 48), rather than a codominant one (*T/T* compared with *G/T* compared with *G/G*), as was used in the current study. Results opposite of those in the current study were reported from a German and a French study. The German study found the *G* allele to be associated with higher BMI, waist-to-hip ratio, and %BF in those without a family history of diabetes than in those with such a family history (31). In the French study, the *G* allele was associated with increases in body weight and waist-to-hip ratio during a 3-y follow-up (36). Some investigators reported significant associations between the EX2 45T→G − IVS2 276G→T haplotype and measures of obesity (32, 39, 49), but we did not find evidence for such an association.

The *ADIPOQ* IVS2 276G→T variant is not located in a coding region, and its functional implications should be investigated further. Despite the lack of direct evidence that this variant affects the expression concentrations of adiponectin, it was found to be associated with adiponectin concentrations in several populations (40, 44, 45, 47, 48, 50). We found no associations between this *ADIPOQ* gene variant and RMR, RQ, or any of the adiposity-related phenotypes, whereas significant associations with body fat have been reported in African Americans (35) and whites (48).

To the best of our knowledge, only one other study (9) has examined the association between the *ADIPOQ* EX2 45G→T and IVS2 276G→T gene variants and EE and RQ. This Finnish study found no associations in 158 offspring of type 2 diabetic patients (9), which is similar to the findings of the current study.

Several recent studies reported that variants in the proximal promoter region of the *ADIPOQ* gene are associated, either independently or as part of a haplotype, with adiponectin concentrations, type 2 diabetes, and BMI (33, 36, 39, 43, 44, 47). However, we found no association between the *ADIPOQ* −3971A→G variant, located in the 5'-UTR, and any of the adiposity-related phenotypes, which is in accordance with other studies (33, 44). However, we did find a significant genotype-by-genotype interaction between the *ADIPOQ* −3971A→G and *ADIPOR1* −3882T→C variants in relation to RQ and adiposity. The carriers of the *G* allele for the *ADIPOQ* variant who also were *C/C* homozygotes for the *ADIPOR1* SNP had a significantly higher RQ and significantly higher measures of overall and abdominal adiposity than did subjects with the other genotype combinations. The association with substrate oxidation rates was independent of body composition because we adjusted RQ for fat mass and fat-free mass. The lower fat oxidation rates and greater adiposity suggest that this rather rare genotype combination results in less efficient adiponectin-adiponectin receptor signaling than do the other genotypes. However, the molecular interactions between adiponectin and its receptors and the importance of the gene promoter regions in these interactions remain to be elucidated.

The adiponectin receptors mediate increases in AMP-activated protein kinase, peroxisome proliferator–activated receptor α ligand activities, glucose uptake, and fatty acid oxidation by adiponectin (51). To the best of our knowledge, the current study is the first to report that variants in the 5'-UTRs of both *ADIPOR1* (IVS1 −3882T→C) and *ADIPOR2* (IVS1 −1352G→A) are associated with RQ and that the rare homozygotes have a lower rate of fat oxidation (ie, higher RQ). However, none of the *ADIPOR* variants were associated with EE or any of the adiposity-related phenotypes. This finding is in agreement with other studies, which focused on the association between *ADIPOR1* and *ADIPOR2* SNPs in relation to type 2 diabetes and insulin resistance and reported no association with BMI (15–17). Because this is the first study to show an association between *ADIPOR* variants and RQ, further studies are warranted in other populations, and functional studies of these *ADIPOR* gene variants are necessary to clarify their potential roles in substrate oxidation rates and lipid metabolism.

In conclusion, these findings suggest that the *ADIPOQ* 45T→G variation contributes to variation in overall adiposity and abdominal fatness, whereas variants in the proximal promoter regions of *ADIPOR1* and *ADIPOR2* contribute to variation in relative substrate oxidation rates as evidenced by RQ measurements. In addition, a significant genotype-by-genotype interaction was found between gene variants in the proximal promoters of *ADIPOQ* and *ADIPOR1*, and that affects RQ and total body fatness and abdominal adiposity.

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