Interactions Among the α2-, β2-, and β3-Adrenergic Receptor Genes and Obesity-Related Phenotypes in the Quebec Family Study

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The gene-gene interactions between markers in the α2-, β2-, and β3-adrenergic receptor (ADR) genes and obesity-related phenotypes were studied in the Quebec Family Study (QFS) cohort. The prevalence of the Arg allele of the Arg16Gly polymorphism in the β2-ADR gene was higher (49%) in males with a body mass index (BMI) of 35 kg/m² or higher versus those with a BMI less than 35 kg/m² (33%; *P* = .010). The β2-ADR gene Arg16Gly and Gln27Glu polymorphisms were associated with plasma total and low-density lipoprotein (LDL) cholesterol concentrations. In addition, the homozygotes for the 6.3-kb allele of DraI polymorphism in the α2-ADR gene had the lowest mean abdominal subcutaneous fat area (*P* = .012) and total fat area (*P* = .003), as well as insulin area, under the curve during an oral glucose tolerance test ([OGTT] *P* = .004). Several ADR gene-gene interaction effects on abdominal fat distribution and plasma lipids were detected. First, significant interactions between α2- and β3-ADR genes were observed on total (*P* = .015) and subcutaneous (*P* = .004) abdominal fat. Second, interaction effects between α2- and β2-ADR gene variants influenced total, high-density lipoprotein (HDL), and LDL cholesterol concentrations. Finally, there were interactions between markers within the β2-ADR gene affecting plasma triglyceride concentrations and subcutaneous abdominal fat. From these results, we conclude that polymorphisms in the ADR genes contribute to body fat and plasma lipid variability in men. Gene-gene interactions among the ADR genes contribute to the phenotypic variability in abdominal obesity and plasma lipid and lipoprotein, but not in visceral fat levels.

OBESITY is a complex phenotype resulting from the combined effects of genes, behavioral factors, and their interactions.[1] The biological factors that regulate energy balance are of particular importance for human obesity. The role of the adrenergic system in the regulation of energy balance through the stimulation of both thermogenesis and lipolysis in brown and white adipose tissue has long been recognized.[2,3] Human adipocytes harbor adrenergic receptors (ADRs) that can stimulate (β1, β2, and β3)[3] or inhibit (α2)[4] lipolysis. Thus, the ADR genes are reasonable candidate genes for obesity and its comorbidities.

Indeed, associations between polymorphisms at codon 27 of the β2-ADR gene and obesity[5,6] and at codon 16 and hypertriglyceridemia and diabetes mellitus[7] have been reported. Studies on the role of the Trp64Arg polymorphism of the β3-ADR in human obesity have been inconsistent, and its role in obesity may be weak.[8] Less research has been performed on the α2-ADR gene, but an association of a DraI polymorphism with body fat distribution has been reported.[9]

In the present study, we investigated the main effects and the interactions among polymorphisms in the α2-, β2-, and β3-ADR genes on obesity-related phenotypes.

SUBJECTS AND METHODS

The Quebec Family Study (QFS) cohort has been previously described.[10] Briefly, a total of 1,628 individuals from French-Canadian families living in and around Quebec City were recruited through the media during the years 1978 to 1981 (phase 1). Seven hundred forty-three subjects from 194 families participating in phase 2 of the QFS were included in the present study.

Phenotype Measurements

The body mass index (BMI) was calculated as the body weight in kilograms divided by the squared height in meters. Body density obtained by underwater weighing[11] was converted to percent body fat using the equation of Siri[12] with residual pulmonary volume measured by the helium dilution method.[13] Fat mass and fat-free mass were obtained from the percent body fat and body weight. Skinfolds were measured on the left side of the body according to the recommendations of the International Biological Program[14] and included 6 sites: biceps, triceps, medial calf, subscapular, suprailiac, and abdominal. The sum of the 6 skinfold measures was considered as an indicator of total subcutaneous fat. Following a 12-hour overnight fast, venous blood samples for plasma lipid and lipoprotein determinations were obtained with EDTA tubes. Cholesterol and triglyceride concentrations were determined in plasma and lipoprotein fractions using an RA-1000 automated analyzer (Technicon Instruments, Tarrytown, NY) as reported previously.[15] Plasma very–low-density lipoproteins ($d < 1.006$ g/mL) were isolated by ultracentrifugation,[16] and the high-density lipoprotein (HDL) fraction was obtained after precipitation of low-density lipoprotein (LDL) in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$ as previously described.[17]

A 75-g oral glucose tolerance test (OGTT) was performed in the morning after a 12-hour fast. Fasting plasma insulin was determined by radioimmunoassay[18] and plasma glucose by an enzymatic method.[19] Plasma insulin levels were determined at 15-minute intervals during the first hour following glucose ingestion and every 30 minutes for the subsequent 2 hours. The total areas under the curve during the OGTT for insulin and glucose were computed from the plasma levels using the trapezoidal method as previously described.[20] Total abdominal and visceral fat areas were obtained using computed tomography as previously described.[21]

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DNA Analysis

Genomic DNA was isolated from lymphoblastoid cell cultures[22] by digestion with proteinase K and extraction with phenol chloroform.

Polymerase chain reaction analysis. The T to C transition at codon 64 in the β3-ADR gene leads to a replacement of tryptophan by arginine and generates a new MspI restriction site. Specific primers covering this MspI restriction site were generated as reported previously.[23] Each 10-μL reaction contained 250 ng genomic DNA, 0.3 μmol/L of each primer, 0.2 mmol/L of each of the dNTPs, and 2.5 U Taq DNA polymerase in a standard buffer and Q solution (Qiagen, Mississauga, Ontario, Canada). The reactions were incubated at 94°C for 3 minutes, 50°C for 1 minute, and 72°C for 1 minute, followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds and 1 cycle at 72°C for 10 minutes using a thermal cycler (model 9600; Perkin-Elmer Cetus Instruments, Branchburg, NJ). Polymerase chain reaction (PCR) products were digested by adding 7.5 U MspI enzyme for 18 hours at 37°C, and the obtained fragments were separated on a 3% agarose gel and visualized under UV light after staining with ethidium bromide. The manufacturer of the restriction enzymes is New England Biolabs (Mississauga, Ontario, Canada) unless specified otherwise.

PCR analysis of the Gln27Glu polymorphism of the ADR β2 gene was performed in a vol of 20 μL containing 150 ng DNA, 0.3 μmol/L of each primer, 0.2 mmol/L of each of the dNTPs, 1.0 U Taq polymerase, and 1× standard buffer plus 10% dimethyl sulfoxide (DMSO). The primers were those reported earlier.[5] The PCR was started at 95°C for 3 minutes, 60°C for 1 minute, and 72°C for 1 minute, followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds and 1 cycle at 72°C for 10 minutes. The amplified product was digested at 37°C for 18 hours with 1 U ItaI (Roche Diagnostics, Laval, Quebec, Canada). The fragments were separated on 2.0% agarose gel and visualized under UV light after staining with ethidium bromide. The PCR analysis for the Arg16Gly polymorphism of the β2-ADR gene was performed as for the Gln27Glu polymorphism but without DMSO. The primers were those reported earlier.[5] The PCR was started at 95°C for 3 minutes, 57°C for 1 minute, and 72°C for 1 minute, followed by 37 cycles at 95°C for 30 seconds, 57°C for 45 seconds, and 72°C for 45 seconds and 1 cycle at 72°C for 10 minutes. The amplified product was digested at 60°C for 4.5 hours with 4 U BsrDI. The fragments were separated on 3.0% agarose gel and visualized under UV light after staining with ethidium bromide.

Southern blot analysis. The human α2- and β2-ADR genomic probes were obtained from the American Type Culture Collection (Rockville, MD). Five micrograms of genomic DNA was digested 18 to 20 hours with 30 U DraI for α2-ADR and BanI restriction enzyme for β2-ADR according to conditions recommended by the manufacturer. DNA fragments were separated on a 1.2% agarose gel by electrophoresis in 1× TEA buffer (40 mmol/L Tris-acetate and 1 mmol/L EDTA, pH 8.0) and transferred by alkali blotting[24] in 0.25 mol/L NaOH and 1.5 mol/L NaCl to nylon membrane (Hybond N+; Amersham Pharmacia Biotech, Uppsala, Sweden). The probes were labeled with [^{32}P] to a specific activity of 1 to 2 × 10^9 dpm/μg using random priming.[25]

Prehybridization was performed in 3 mL solution containing 1 mmol/L EDTA, 0.25 mol/L Na_2HPO_4 (pH 7.4), 7% sodium dodecyl sulfate (SDS), and 100 μg/mL denatured salmon sperm DNA for a minimum of 4 hours at 65°C in a hybridization incubator (Robbins Scientific, Sunnyvale, CA). The filters were hybridized with the [^{32}P]-labeled probes for 18 hours in 1.5 mL of the same solution. They were then washed for 20 minutes 3 times in 0.1 × SSPE (20 mmol/L NaCl, 1 mmol/L NaH_2PO_4, and 0.1 mmol/L EDTA, pH 7.7) and 0.1% SDS at 65°C. Fragments were visualized by autoradiography using Kodak XAR-5 film with intensifying screens at −70°C for 3 to 5 days (Eastman Kodak, Rochester, NY). The size of the fragments was estimated using λDNA digested with EcoRI and HindIII as standard.

Statistical Analysis

All analyses were performed with the SAS Statistical Software Package (SAS Institute, Cary, NC). A chi-square test was performed to assess whether the observed genotype frequencies were in Hardy-Weinberg equilibrium. Differences in allele and genotype frequencies between men and women and between BMI classes were also assessed by χ^2 tests. Linkage disequilibrium between markers was assessed as described by Terwilliger and Ott.[26] Associations between the gene markers and phenotypes were tested using the general linear model (GLM) procedure. The body weight, BMI, fat mass, fat-free mass, and sum of 6 skinfolds were adjusted for age and sex. Plasma lipid and lipoprotein and plasma insulin concentrations were adjusted for total fat mass, age, and sex, and for abdominal visceral fat for some analyses. Because of the skewed distribution, insulin values were log(10)-transformed and 95% confidence intervals (CIs) are given. Abdominal fat areas were adjusted for total fat mass, age, and sex. Gene-gene interactions were tested with the GLM procedure by including the gene main effects, interaction terms, and covariates in the same model.

All of the family members were included in the analyses. Although it is commonly believed that the relatedness of the subjects in family study cohorts may cause problems in association analyses, a recent simulation study (M. Province, T. Rice, D.C. Rao, unpublished data, 1999) suggests that this is not the case. In that study, the data were analyzed by 4 methods, and the least-squares method used in the present report was one of them; the other 3 methods treated dependencies in different ways. The results show that, first, the failure to incorporate dependencies did not induce any bias and, second, for moderate familial correlations as found in most family studies (including the current one), ignoring the dependencies by using ANOVA performed quite well. The only negative impact was a small reduction in power. The standard errors were slightly enlarged but, most importantly, type I error was unaffected. Given this, we do not believe that the dependencies or relatedness of the subjects in families cause any real problems in this type of analysis.

RESULTS

Phenotypic characteristics of the 743 subjects are shown in Table 1. Men were heavier and had a lower fat mass and higher fat-free mass, lower total subcutaneous fat and total and subcutaneous abdominal fat, but higher abdominal visceral fat compared with women.

The genotype distribution and allele frequency of the 5 polymorphisms based only on parents are presented in Table 2. There were no significant differences between the males and

Table 1. Characteristics of the Subjects of the QFS Cohort (N = 743)

Variable	Males		Females	
	Mean ± SEM	No.	Mean ± SEM	No.
Age (yr)	42.5 ± 0.9	322	42.8 ± 0.8	421
Body weight (kg)	81.1 ± 1.1	320	69.8 ± 1.0*	417
BMI (kg/m^2)	27.2 ± 0.3	320	27.4 ± 0.4	417
Fat mass (kg)	19.6 ± 0.7	281	23.5 ± 0.8*	336
Fat-free mass (kg)	60.9 ± 0.5	281	45.5 ± 0.4*	336
Subcutaneous fat (mm, sum of 6 skinfolds)	96.5 ± 3.0	312	143.4 ± 3.6*	390
Abdominal total fat area (cm^2)	342.1 ± 12.9	223	425.7 ± 13.4*	292
Abdominal visceral fat area (cm^2)	126.5 ± 5.5	223	97.9 ± 3.8*	292
Abdominal subcutaneous fat area (cm^2)	215.6 ± 8.8	223	327.8 ± 10.6*	292

*$P < .0005$, males *v* females.

Table 2. Genotype and Allele Frequencies for the α2-, β2-, and β3-ADR Polymorphisms (only parents included)

Gene/Polymorphism	Genotype Frequency			Allele Frequency	
α2-ADR/DraI	6.7/6.7 kb	6.7/6.3 kb	6.3/6.3 kb	6.7 kb	6.3 kb
	0.70 (232)	0.27 (89)	0.03 (10)	0.83	0.17
β2-ADR/Gln27Glu	Gln27Gln	Gln27Glu	Glu27Glu	Gln	Glu
	0.38 (128)	0.41 (135)	0.21 (71)	0.59	0.41
β2-ADR/BanI	3.7/3.7 kb	3.7/3.4 kb	3.4/3.4 kb	3.7 kb	3.4 kb
	0.04 (13)	0.30 (99)	0.66 (223)	0.19	0.81
β2-ADR/Arg16Gly	Arg16Arg	Arg16Gly	Gly16Gly	Arg	Gly
	0.17 (57)	0.39 (128)	0.44 (148)	0.36	0.64
β3-ADR/Trp64Arg	Trp64Trp	Trp64Arg	Arg64Arg	Trp	Arg
	0.85 (282)	0.15 (51)	0.00 (1)	0.92	0.08

NOTE. The number of subjects is shown in parentheses.

females in genotype or allele frequencies (data not shown). All genotype frequencies were in Hardy-Weinberg equilibrium. Allelic frequencies were comparable to those reported previously for all markers.[5,6,23,27,28]

Strong linkage disequilibrium was observed between the β2-ADR markers (χ^2 = 122.74 to 441.08, $P < .001$ for all).

When allele frequencies for the α2-, β2-, and β3-ADR polymorphisms among BMI groups (cutoff, 35 kg/m^2) were compared, there were no differences except for 1 case. The prevalence of the Arg allele of the Arg16Gly polymorphism in the β2-ADR gene was higher (χ^2 = 6.61, P = .010, df = 1) in males with a BMI of 35 kg/m^2 or higher (49.0%, n = 35) versus those with a BMI less than 35 kg/m^2 (33.0%, n = 189). When lower cutoff points for BMI classification were used (25 or 30 kg/m^2), no significant differences in allele frequencies were observed (data not shown).

α2-ADR Gene DraI Polymorphism

In the whole group, homozygotes for the 6.3-kb allele of the α2-ADR DraI polymorphism had the lowest abdominal subcutaneous and total fat areas (Fig 1). The α2-ADR DraI genotype explained 0.2% of the variation in total and subcutaneous abdominal fat areas. The insulin area under the curve during the OGTT was the lowest (47.30×10^3 pmol/L · min, 95% CI = 38.90 to 58.50, P for trend = .004) in 6.3-kb homozygotes compared with homozygotes for the 6.7-kb allele (67.70×10^3 pmol/L · min, 95% CI = 64.90 to 70.50, P = .001 *v* 6.3/6.3-kb genotype) or the heterozygotes ($66.30 \cdot 10^3$ pmol/L · min, 95% CI = 62.3 to 72.0, P = .002 *v* 6.3/6.3-kb genotype). The difference remained almost the same after adjustment for abdominal visceral fat. The α2-ADR DraI genotype explained 1.6% of the variation in insulin area. The glucose areas did not differ between DraI genotypes.

β2-ADR Gene Polymorphism

In the case of the Arg16Gly polymorphism of the β2-ADR gene, plasma cholesterol was higher (P for trend = .027) in subjects who were homozygous for the Gly allele compared with homozygotes for the Arg allele in the whole cohort and in men (Table 3). A similar difference was observed for LDL cholesterol in men, but not in women. When adjustments for abdominal visceral fat were made, the differences in cholesterol concentrations were slightly greater. Gly allele homozygotes also tended to have the lowest BMI (P for trend = .051, data not shown).

As for the Gln27Glu polymorphism of the β2-ADR gene, plasma cholesterol was lower (P for trend = .016) in homozygotes for the Gln allele versus heterozygotes and homozygotes for the Glu allele. A similar difference was also found for LDL cholesterol in men (Table 3). When adjustments for abdominal visceral fat were made, the differences in cholesterol were slightly greater. The β2-ADR markers explained 0.9% to 2.9% of the variation in total cholesterol and 2.2% to 2.5% of the variation in LDL cholesterol.

β3-ADR Gene Polymorphism

Male carriers of the Arg allele of the β3-ADR Trp64Arg polymorphism (n = 29) had a larger amount of total (P = .018) and subcutaneous (P = .029) abdominal fat (363.0 ± 11.0 and 235.4 ± 10.0 cm^2, respectively) than the Trp homozygotes (334.8 ± 4.3 and 211.8 ± 3.9 cm^2, n = 184). No other differences were found for this marker.

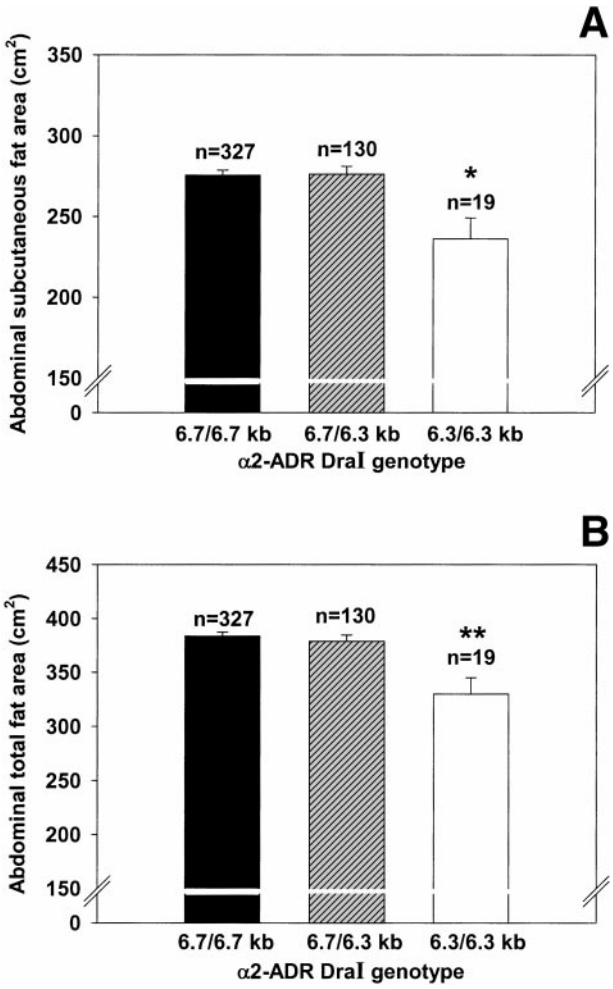

Fig 1. Mean abdominal fat area by α2-ADR DraI genotype in the whole group (mean ± SEM) adjusted for age, sex, and total fat mass. *P* for trend = .012 for A and .003 for B. **P* = .003, 6.3/6.3 kb *v* 6.7/6.7 kb; *P* = .004, 6.3/6.3 kb *v* 6.7/6.3 kb. ***P* = .001, 6.3/6.3 kb *v* 6.7/6.7 kb; *P* = .003 6.3/6.3 kb *v* 6.7/6.3 kb.

Table 3. Plasma Lipids in Relation to β2-ADR Genotypes in the Whole Group and in Men

Polymorphism	Group	Total Cholesterol	LDL Cholesterol	HDL Cholesterol	Total Triglycerides
β2-ADR/Arg16Gly, whole cohort					
Arg16Arg (n = 69)	I	4.81 ± 0.11	2.97 ± 0.09	1.19 ± 0.03	1.47 ± 0.18
Arg16Gly (n = 279)	II	4.98 ± 0.05	3.10 ± 0.04	1.24 ± 0.02	1.47 ± 0.09
Gly16Gly (n = 264)	III	5.12 ± 0.06*	3.19 ± 0.05	1.24 ± 0.02	1.63 ± 0.09
P for trend		.027	NS	NS	NS
β2-ADR/Arg16Gly, men					
Arg16Arg (n = 31)	I	4.72 ± 0.15	2.99 ± 0.13	1.05 ± 0.05	1.53 ± 0.16
Arg16Gly (n = 121)	II	4.98 ± 0.07	3.20 ± 0.06	1.09 ± 0.02	1.55 ± 0.08
Gly16Gly (n = 129)	III	5.22 ± 0.07†	3.38 ± 0.06‡	1.12 ± 0.02	1.71 ± 0.08
P for trend		.003	.013	NS	NS
β2-ADR/Gln27Glu, whole cohort					
Glu27Glu (n = 110)	I	5.12 ± 0.09	3.25 ± 0.07	1.27 ± 0.03	1.38 ± 0.14
Gln27Glu (n = 286)	II	5.10 ± 0.05	3.14 ± 0.04	1.24 ± 0.02	1.65 ± 0.09
Gln27Gln (n = 217)	III	4.88 ± 0.06§	3.04 ± 0.05	1.20 ± 0.02	1.47 ± 0.10
P for trend		.016	NS	NS	NS
β2-ADR/Gln27Glu, men					
Glu27Glu (n = 53)	I	5.31 ± 0.11‖	3.50 ± 0.10¶	1.14 ± 0.03	1.59 ± 0.12
Gln27Glu (n = 97)	II	5.10 ± 0.07	3.27 ± 0.06	1.10 ± 0.02	1.67 ± 0.08
Gln27Gln (n = 130)	III	4.89 ± 0.08	3.12 ± 0.07	1.08 ± 0.03	1.59 ± 0.09
P for trend		.009	.008	NS	NS

NOTE. Values are the mean ± SEM, with the number of subjects in parentheses.
*P = .013, III *v* I.
†P = .002, III *v* I; P = .020, III *v* II.
‡P = .007, III *v* I.
§P = .030, III *v* I; P = .009, III *v* II.
‖P = .003, I *v* III.
¶P = .046, I *v* II; P = .002, I *v* III.

Gene-Gene Interactions

A significant interaction was observed between the α2-ADR and β3-ADR gene markers for abdominal fat, with noncarriers of the 6.3-kb allele of the α2-ADR DraI and carriers of the Arg allele of the β3-ADR Trp64Arg polymorphisms having the highest amount of total (P = .015 for the α2-ADR × β3-ADR marker interaction; Fig 2) and subcutaneous abdominal fat (P = .004 for the α2-ADR × β3-ADR marker interaction; data not shown). This interaction was also found in men (P = .015 for α2-ADR × β3-ADR marker interaction) but not in women: in males, noncarriers of the 6.3-kb allele and carriers of the Arg allele had more abdominal subcutaneous fat (258.9 ± 13.6 cm^2, mean ± SEM, n = 15) compared with carriers of the 6.3-kb allele and Arg allele (207.7 ± 14.2 cm^2, P = .010, n = 14), noncarriers of the 6.3-kb allele and Arg allele (211.2 ± 4.8 cm^2, P = .001, n = 122), or carriers of the 6.3-kb allele and noncarriers of the Arg allele (212.5 ± 6.7 cm^2,P = .002, n = 61).

Figure 3 and Table 4 show the characteristics for the 2

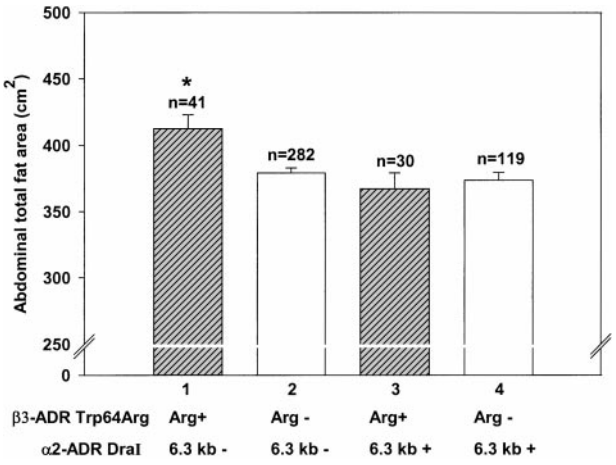

Fig 2. Mean abdominal total fat in relation to β3- and α2-ADR genotypes in the whole group (mean ± SEM). For the β3- × α2-ADR marker interaction, P = .015. *P = .002, 1 *v* 2; P = .004, 1 *v* 3; P = .001, 1 *v* 4.

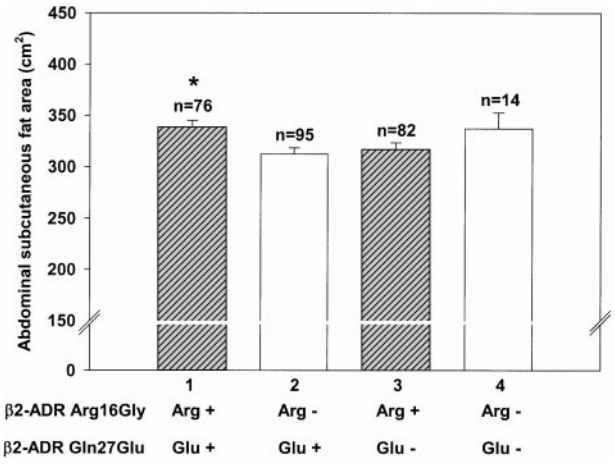

Fig 3. Mean abdominal subcutaneous fat area in relation to the β2-ADR Arg16Gly and Gln27Glu genotypes in women (mean ± SEM). For the β2-ADR marker interaction, P = .016. *P = .004, 1 *v* 2; P = .021, 1 *v* 3.

Table 4. Interactions Between Arg16Gly and BanI Polymorphisms of the β2-ADR Gene for the Obesity-Related Phenotypes in 320 Males of the QFS

Parameter	β2-ADR BanI				*P* for Interaction
	3.4-kb Allele Noncarriers		3.4-kb Allele Carriers		
	(I) Arg Allele Carriers	(II) Arg Allele Noncarriers	(III) Arg Allele Carriers	(IV) Arg Allele Noncarriers	
No. of subjects	49	72	134	65	
BMI (kg/m^2)	25.9 ± 0.9	26.8 ± 0.7	28.6 ± 0.5*	25.9 ± 0.7	.012
Fat mass (kg)	18.2 ± 1.8	19.3 ± 1.4	21.7 ± 1.1	17.1 ± 1.5	NS
Sum of 6 skinfolds (mm)	92.3 ± 7.5	92.9 ± 6.2	105.7 ± 4.6	85.5 ± 6.5	NS
Abdominal total fat area (cm^2)	338.1 ± 10.9	331.9 ± 8.0	345.4 ± 6.6	334.5 ± 8.8	NS
Abdominal visceral fat (cm^2)	118.2 ± 7.6	118.7 ± 5.6	132.4 ± 4.6	117.4 ± 6.1	NS
Abdominal subcutaneous fat (cm^2)	220.7 ± 9.9	213.2 ± 7.3	213.0 ± 6.0	217.1 ± 8.0	NS
Total cholesterol (mmol/L)	5.08 ± 0.13	5.15 ± 0.10	4.87 ± 0.08	5.30 ± 0.10	NS
LDL cholesterol (mmol/L)	3.30 ± 0.11	3.31 ± 0.09	3.11 ± 0.07	3.45 ± 0.09	NS
HDL cholesterol (mmol/L)	1.13 ± 0.04	1.07 ± 0.03	1.06 ± 0.02	1.17 ± 0.03†	.011
Total triglycerides (mmol/L)	1.47 ± 0.14	1.81 ± 0.11	1.58 ± 0.08	1.60 ± 0.11	NS

NOTE. Values are the mean ± SEM.

*P = .008, III *v* I; P = .048, III *v* II; P = .003, III *v* IV.

†P = .036, IV *v* II; P = .010, IV *v* III.

polymorphisms of the β2-ADR gene. Female carriers of the Arg allele of the β2-ADR Arg16Gly polymorphism and carriers of the Glu allele of the β2-ADR Gln27Glu polymorphism (group 1) had a higher amount of abdominal subcutaneous fat than noncarriers of Arg and carriers of Glu alleles (P = .004) or carriers of Arg and noncarriers of Glu alleles (P = .021; Fig 3). The female noncarriers of both the Arg allele and Glu allele had a similar profile as group 1, but it was not significant. Male subjects carrying both the Arg allele of the Arg16Gly polymorphism and the 3.4-kb allele of the BanI polymorphism had the highest BMI and the lowest HDL cholesterol and tended to have the lowest LDL and total cholesterol (Table 4). Adjustment for abdominal visceral fat did not change the differences in lipid concentrations (data not shown).

Significant associations between genetic variants and lipid values are shown in Table 5. A significant interaction was observed between the α2-ADR and β2-ADR genes for HDL cholesterol for all marker combinations (α2-ADR/DraI and β2-ADR/BanI, α2-ADR/DraI and β2-ADR/Gln27Glu, and α2-ADR/DraI and β2-ADR/Arg16Gly). The interaction was also significant for total and LDL cholesterol between α2-ADR and β2-ADR genes and for triglycerides between β2-ADR Arg16Gly and BanI polymorphisms (Table 5). The interaction between the α2-ADR and β3-ADR genes explained 1.5% and 0.2% of the variation in total and subcutaneous abdominal fat areas, respectively. The α2-ADR and β2-ADR gene interactions explained 0.5% to 0.8% of the variation in HDL cholesterol concentrations.

DISCUSSION

Our data demonstrate associations between the α2-ADR DraI polymorphism and the abdominal total and subcutaneous fat areas. The β2-ADR Arg16Gly and Gln27Glu polymorphisms are also associated with plasma cholesterol concentrations in the whole cohort, and the Arg16Gly polymorphism is associated with obesity (BMI $>$ 35 kg/m^2) in men. The results also suggest multiple gene-gene interactions in the ADR gene family that affect abdominal fat levels and plasma lipids. These observations were particularly consistent in men.

In the current study, no significant patterns for the variation in abdominal fat with the polymorphisms of β2- or β3-ADR genes were observed, although the β3-ADR Trp64Arg polymorphism was weakly associated with total and subcutaneous abdominal fat in men. However, the α2-ADR locus was associated with the abdominal total and particularly abdominal subcutaneous fat areas, with homozygotes for the 6.3-kb allele of the DraI polymorphism having the lowest values. In contrast, in a previous study, this allele was associated with an increase in the ratio of trunk to extremity skinfolds in women.[9] Our series showed that this genotype was also associated with the lowest insulin area under the curve during the OGTT. Since there was no difference in the glucose area between genotypes, the effect of the 6.3-kb allele on insulin metabolism may occur via a reduction of insulin resistance and is probably a peripheral effect. The association remained significant after adjustment for abdominal visceral fat, which is consistent with the notion that it is not dependent on the amount of visceral fat. This variant may thus have some protective effects against the metabolic complications associated with obesity. However, whether the DraI variant causes a change in the sequence of the gene product and whether it has functional significance remain unknown. Also, the exact site of this marker is yet unknown, although recent data suggest the variant is unlikely to be within the coding region of the gene.[29] Therefore, it could be in linkage disequilibrium with a nearby locus that has functional significance.

In our study cohort, the findings were more clear in men. In prior studies, different effects of the Gln27Glu polymorphism of the β2-ADR gene in men and women have been observed.[5,6] We did not find any association of this polymorphism on regional fat distribution or obesity in men or women. However, the prevalence of the Gly16 allele of the Arg16Gly polymorphism of β2-ADR was lower in men with a BMI greater than 35 kg/m^2 versus those with a BMI less than 35 kg/m^2. In a Japanese population,[7] a similar difference in Gly16 between obese (BMI $>$ 27 kg/m^2) and non-obese (BMI $<$ 27 kg/m^2) women was observed. In our study, men who were homozygous for the Gly allele tended to have the lowest BMI but the highest plasma

Table 5. Plasma Lipids in Relation to the Genotypes at Two ADR Genes in the Whole Cohort

Polymorphisms		No.	Group	Total Cholesterol	LDL Cholesterol	HDL Cholesterol	Total Triglycerides
α2 DraI	β2 BanI						
6.3-kb allele	3.4-kb allele						
−	+	265	I	4.98 ± 0.06	3.08 ± 0.05	1.26 ± 0.02	1.45 ± 0.09
−	−	160	II	5.08 ± 0.07	3.15 ± 0.06	1.18 ± 0.02*	1.75 ± 0.12
+	+	115	III	5.04 ± 0.09	3.17 ± 0.07	1.22 ± 0.03	1.46 ± 0.14
+	−	71	IV	5.02 ± 0.11	3.13 ± 0.09	1.26 ± 0.03	1.48 ± 0.18
P for interaction				NS	NS	.013	NS
α2 DraI	β2 Gln27Glu						
6.3-kb allele	Glu allele						
−	+	272	I	5.12 ± 0.06	3.17 ± 0.05	1.24 ± 0.02	1.64 ± 0.09
−	−	149	II	4.86 ± 0.08	3.01 ± 0.06	1.22 ± 0.02	1.43 ± 0.12
+	+	120	III	5.09 ± 0.08	3.18 ± 0.07	1.28 ± 0.02	1.41 ± 0.14
+	−	65	IV	4.94 ± 0.11	3.11 ± 0.09	1.15 ± 0.03†	1.59 ± 0.18
P for interaction				NS	NS	.037	NS
α2 DraI	β2 Arg16Gly						
6.3-kb allele	Arg allele						
−	+	237	I	4.90 ± 0.06	3.02 ± 0.05§	1.24 ± 0.02	1.44 ± 0.10
−	−	183	II	5.18 ± 0.07‡	3.23 ± 0.06	1.22 ± 0.02	1.72 ± 0.11
+	+	106	III	5.06 ± 0.09	3.20 ± 0.07	1.19 ± 0.03‖	1.52 ± 0.14
+	−	79	IV	5.00 ± 0.10	3.10 ± 0.09	1.29 ± 0.03	1.41 ± 0.17
P for interaction				.034	.019	.012	NS
β2 BanI	β2 Arg16Gly						
3.4-kb allele	Arg allele						
−	+	92	I	4.94 ± 0.10	3.10 ± 0.08	1.21 ± 0.03	1.41 ± 0.15
−	−	137	II	5.16 ± 0.08	3.18 ± 0.06	1.20 ± 0.02	1.86 ± 0.13¶
+	+	254	III	4.95 ± 0.06	3.06 ± 0.05	1.23 ± 0.02	1.49 ± 0.09
+	−	127	IV	5.09 ± 0.08	3.21 ± 0.07	1.28 ± 0.02	1.39 ± 0.13
P for interaction				NS	NS	NS	.033

NOTE. Values are the mean ± SEM. +, carriers; −, noncarriers of the allele.
*$P = .002$, II *v* I; $P = .041$, II *v* IV.
†$P = .021$, IV *v* I; $P = .003$, IV *v* III.
‡$P = .002$, II *v* I.
§$P = .004$, I *v* II; $P = .043$, I *v* III.
‖$P = .018$, III *v* IV.
¶$P = .025$, II *v* I; $P = .019$, II *v* III; $P = .010$, II *v* IV.

cholesterol. The fact that adjustment for abdominal visceral fat slightly increased this effect on plasma lipids supports the hypothesis that it is independent of visceral fat. The Gln27Glu polymorphism, which is in tight linkage disequilibrium with the Arg16Gly polymorphism, also had a clear association with plasma total cholesterol. The β2-ADR Gln27Glu and Arg16Gly polymorphisms have been shown to be associated with altered β2-ADR function in recombinant cells,[30,31] and the Arg16Gly polymorphism is associated with altered native human β2-ADR function in women,[5] although it has not been studied in men. In the study by Large et al,[5] female Gly16 allele carriers showed a 5-fold increase in agonist sensitivity.

ADR genes do not appear to be major genes affecting abdominal fat distribution, particularly abdominal visceral fat. However, the data of the present study show that the interactions between and within loci of the α2-, β2-, and β3-ADR genes are associated with variations in abdominal fat accumulation and other obesity-related phenotypes, but not with visceral fat levels. Several ADR gene-gene interaction effects on abdominal fat distribution and plasma lipids were detected. First, the interactions between the α2-ADR and β3-ADR genes on abdominal total and subcutaneous fat distribution were characterized by an association between the β3-ADR polymorphism and abdominal fat phenotype that was found only in noncarriers of the 6.3-kb allele of the α2-ADR DraI polymorphism. These interaction effects between α2-ADR and β3-ADR loci may be one of the factors that could explain the discrepancies in results on the effects of the β3-ADR Trp64Arg polymorphism in various populations. Second, interactions between the α2- and β2-ADR genes influenced plasma HDL cholesterol concentrations. The effect of the α2- and β2-ADR genes on HDL cholesterol was not observed for the gene markers analyzed individually. Finally, there were interactions between the 2 markers within the β2-ADR gene affecting abdominal subcutaneous fat in women. How could gene-gene interactions within the ADR system increase the accumulation of fat in abdominal depots? It is possible that the α2- and β2-ADR gene interactions influence the functional balance between α2- and β2-ADR activities, which are strong determinants of the net effect of catecholamines on lipolysis.[32] This might lead to decreased lipolysis and accumulation of fat in the abdominal region.

Some other polymorphic combinations within the ADR genes also had effects on plasma lipid concentrations. For instance,

the combined carrier status of the Arg allele (Arg16Gly polymorphism) and the 3.4-kb allele (BanI polymorphism) within the β2-ADR gene was associated with the highest BMI and the lowest HDL cholesterol, as well as a tendency for the lowest LDL and total cholesterol levels. A haplotype within the β2-ADR gene was associated with plasma triglyceride concentrations. The hypothesized influences of the ADR variants on ADR function and lipolysis could have altered the release of fatty acids and thus may have affected plasma triglyceride concentrations. Carrying 2 variants may alter the functions of the ADR even more. Adjustment of the lipid phenotypes for abdominal visceral fat did not significantly change the patterns of association. Since these analyses were all performed on data adjusted for the fat mass and, when specified, the abdominal visceral fat level, the results provide strong support for the concept that the effects of polymorphisms in the β2-ADR gene are independent of the overall level of fatness and the amount of visceral fat.

In conclusion, the data of this study suggest an association between the α2-ADR DraI polymorphism and the abdominal fat level, as well as plasma insulin. In addition, associations between the β2-ADR Arg16Gly and Gln27Glu polymorphisms and plasma cholesterol in the whole cohort, as well as the Arg16Gly polymorphism and obesity in men, were observed. Most associations with the phenotypes were found with the α2 and β2 markers, and less often with the β3 marker. Several ADR gene-gene interaction effects on abdominal fat levels and plasma lipid values were also detected as contributing to the marked phenotypic variability in abdominal obesity and its comorbidities.

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