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PAPER

The hormone-sensitive lipase gene and body composition: the HERITAGE Family Study

C Garenc[1], L Pérusse[1]*, YC Chagnon[1], T Rankinen[2], J Gagnon[1,3], IB Borecki[4], AS Leon[5], JS Skinner[6], JH Wilmore[7], DC Rao[4,8] and C Bouchard[2]

[1]*Division of Kinesiology, Department of Preventive Medicine, Laval University, Ste-Foy, Québec, Canada;* [2]*Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana, USA;* [3]*Laboratory of Molecular Endocrinology, CHUQ Research Center, CHUL Pavilion, Ste-Foy, Québec, Canada;* [4]*Division of Biostatistics and Department of Genetics, Washington University Medical School, St Louis, Missouri, USA;* [5]*School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, Minnesota, USA;* [6]*Department of Kinesiology, Indiana University, Bloomington, Indiana, USA;* [7]*Department of Health and Kinesiology, Texas A&M University, College Station, Texas, USA; and* [8]*Department of Psychiatry, Washington University Medical School, St Louis, Missouri, USA*

OBJECTIVE: To investigate whether the C-60G polymorphism and other markers in the hormone-sensitive lipase (LIPE) gene are associated with baseline body composition and free-fatty acid (FFA) concentrations measured at rest and during low-intensity exercise in white and black subjects participating in the HERITAGE Family Study.
SUBJECTS: Adult sedentary white (245 men and 258 women) and black (91 men and 185 women) subjects.
MEASUREMENTS: body mass index (BMI); fat mass (FAT); percentage body fat (%FAT); fat-free mass (FATFR); sum of eight skinfolds (SF8); subcutaneous (ASF), visceral (AVF) and total (ATF) abdominal fat areas assessed by CT scan; plasma FFA concentrations measured at rest (FFAR), at a power output of 50 W (FFA50) and at a relative power output of 60% of VO_{2max} (FFA60%); and fasting insulin (INS).
STATISTICAL ANALYSIS: Association between the C-60G polymorphism of the LIPE gene and each phenotype was tested separately in men and women using ANCOVA with the effects of age and race as covariates and with further adjustment for FAT for ASF, AVF, ATF, FFAR, FFA50 and FFA60%. Secondly, owing to significant gene-by-race interaction, associations were investigated separately in each of the two race groups. Linkage was tested with the C-60G polymorphism, a dinucleotide repeat polymorphism in the intron 7 of the LIPE gene and two microsatellites markers (D19S178 and D19S903) flanking the LIPE gene.
RESULTS: There were no race differences in the allele frequencies of the C-60G polymorphism of the LIPE gene. No association or gene-by-race interaction was observed in men. However, in women, strong gene-by-race interactions were observed for BMI ($P = 0.0005$), FAT ($P = 0.0007$), %FAT ($P = 0.0003$), SF8 ($P = 0.0001$), ASF ($P = 0.03$) and ATF ($P = 0.01$). When the analysis was performed separately in each race, white women carriers of the -60G allele exhibited lower %FAT ($P = 0.005$) and SF8 ($P = 0.01$) than non-carriers, while in black women, the -60G allele was associated with higher BMI ($P = 0.004$), FAT ($P = 0.009$), %FAT ($P = 0.01$) and SF8 ($P = 0.0009$). These associations were no longer significant after adjusting for INS. Evidence of linkage was observed in whites with ATF, FFAR, FFA50 and FFA60%.
CONCLUSION: These results suggest that the C-60G polymorphism in the LIPE gene plays a role in determining body composition and that its effect is sex-, race- and insulin-dependent.
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Keywords: hormone-sensitive lipase gene; body composition; abdominal fat; free-fatty acids; association

Introduction

Triacylglycerol is the major fuel stored in adipose tissue and is utilized for energy during exercise and in the fasting state. The hydrolysis of triacylglycerol to 2-monoacylglycerol and two free-fatty acids (FFA) is controlled by hormone-sensitive lipase (HSL). The remaining 2-monoacylglycerol is hydrolyzed into glycerol and FFA by a non-specific mono-acylglycerol lipase. FFAs circulate in the blood bound to albumin and are taken up by different tissues such as liver, skeletal muscle, heart and renal cortex. Glycerol is mainly transported in the blood to be metabolized in the liver. In adipose tissue, HSL activity is controlled by the neural and endocrine

*Correspondence: L Pérusse, Division of Kinesiology, Dept. Preventive Medicine, PEPS-Laval University, Ste-Foy, Québec, Canada G1K 7P4.
E-mail: louis.perusse@kin.msp.ulaval.ca
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systems; the amount of lipid mobilization depends on the balance between the stimulatory or inhibitory pathways.[1–3] During exercise, lipid mobilization is mainly stimulated by increased catecholamine production leading to enhanced β-adrenoceptor-mediated stimulation of lipolysis.[4] Moreover, FFA is a major source of energy for skeletal muscle during low-intensity and prolonged exercise.[5] Impaired function of HSL was proposed to be an early metabolic disturbance leading to the development of obesity.[6] In subcutaneous adipocytes, HSL expression (HSL mRNA, total protein or HSL active enzyme) is a major determinant of the maximal lipolytic capacity[7] and a reduced expression and function is present in obesity.[8]

The gene coding for the HSL, the LIPE gene, maps to the 19q13.1–q13.2 region.[9,10] Several polymorphisms have been found in the LIPE gene with evidence of association with some metabolic dysfunctions. The Arg309Cys polymorphism was associated with fasting serum total cholesterol in Japanese subjects with type 2 diabetes, but not with type 2 diabetes status, obesity and serum triglyceride levels.[11] An association was also found between a dinucleotide repeat in intron 7 of the LIPE gene[12] and type 2 diabetes in subjects with abdominal obesity.[13] Results from two studies revealed no evidence of linkage between the same dinucleotide repeat and familial combined hyperlipidemia in Finnish families[14] or atherogenic lipoprotein phenotype in dizygotic female twins.[15] Magre *et al* reported that the HSLi6$(CA)_n$ repeat polymorphism was associated with type 2 diabetes and obesity.[16] Moreover, sib-pair analysis in French families with morbid obesity showed that LIPE locus is a good candidate for explaining this complex trait.[17] A C-to-G single nucleotide change has been identified[18] at position -60 of the LIPE gene promoter (C$-$60G), but no association between this polymorphism and obesity-related phenotypes has been reported yet.

In the present study, we investigated the associations between the C$-$60G polymorphism of the LIPE gene and body fatness, as well as plasma FFA concentrations measured at rest and during low-intensity exercise. Genetic linkage was also investigated with the C$-$60G polymorphism, as well as with the dinucleotide repeat polymorphism in intron 7 of the LIPE (CA/GT), and the microsatellite markers D19S178 (0.3 cM, centromeric) and D19S903 (0.8 cM, telomeric) flanking the LIPE gene.

Methods

Subjects

The HERITAGE Family Study aims, design and methods have been described elsewhere.[19] All subjects had to be sedentary and in good health and meet a set of inclusion criteria.[19] The population of the present study includes a total of 503 white (245 men and 258 women) and 276 black (91 men and 185 women) adult subjects. The Institutional Review Board at each institution of the HERITAGE Family Study research consortium approved the study protocol. Written informed consent was obtained from each participant.

Phenotype measurements

Anthropometric and body density measurements. These measurements have been described in detail previously.[20] Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). The sum of eight skinfolds (SF8) was used to assess the level of subcutaneous fat. Hydrostatic weighing was used to assess body density. Percentage body fat (%FAT) was estimated from body density as described elsewhere;[20] fat mass (FAT) and fat-free mass (FATFR) were derived.

Abdominal visceral, subcutaneous and total fat areas. Abdominal fat was assessed by computed tomography (CT) as previously described.[21] Scans were obtained between the fourth and fifth lumbar (L4–L5) vertebrae, subjects being in the supine position with arms stretched above the head. Total fat area was calculated using an attenuation range of -190 to -30 Hounsfield units. Abdominal visceral fat (AVF) area was defined by drawing a line within the inner portion of the muscle walls surrounding the abdominal cavity. Abdominal subcutaneous fat area (ASF) was obtained by calculating the difference between total abdominal fat (ATF) and AVF areas.

Exercise tests. Subjects performed an exercise test on a stationary cycle ergometer (Ergo-Metrics 800S, SensorMedics, Yorba Linda, CA, USA). Three exercise tests were performed: the maximal, the submaximal and the submaximal/maximal tests.[22] First, subjects completed a maximal exercise test starting at a power output of 50 W for 3 min with increases of 25 W every 2 min until volitional exhaustion. Secondly, subjects performed a submaximal exercise test during 8–12 min at an absolute power output of 50 W followed by a relative power output equivalent to 60% of their initial VO_{2max} during 8–12 min. Finally, in a submaximal/maximal test, subjects started to exercise with the same protocol as in the submaximal test followed by 3 min of exercise at a power output equivalent to 80% of the initial VO_{2max}. The resistance was then increased to the highest power output attained in the first test and by increasing the power output by 25 W every 2 min thereafter until exhaustion. During the submaximal/maximal test, a venous catheter was inserted in the left arm to obtain blood samples at rest, at 50 W and at 60% of the initial VO_{2max}. Subjects were asked to refrain from eating for at least 2 h before the test.[19] Blood was collected in EDTA tubes, centrifuged at 1000 ***g*** for 10 min at 4°C and plasma was kept frozen at -80°C. Plasma total protein was also measured to correct for the changes in hemoconcentration during the test.

Plasma free-fatty acids and total protein. Plasma FFA at rest (FFAR), 50 W (FFA50) and at 60% of VO_{2max} (FFA60%) were measured using an enzymatic colorimetric method kit

(Wako Chemicals, TX, USA). This procedure was automated on a Technicon RA-XT system. The colorimetric method of Biuret was used to determine plasma total protein.

Fasting plasma insulin. Blood samples for determining plasma insulin concentrations were collected after an overnight fast. Fasting plasma insulin (INS) was measured by radioimmunoassay.[23]

Genotype determination

PCR amplification of the C−60G polymorphism. DNA was extracted from lymphoblastoid cell lines after digestion by proteinase K and purification with phenol-chloroform. PCR amplification was carried out in a volume of 20 μl containing 150 ng DNA, 200 μM each of dATP, dCTP, dGTP and dTTP, 0.9 mM $MgCl_2$, 1X buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH = 7.5 at 25°C), 2X of Q solution, 300 nM of each primer and 1 U of *Taq* polymerase (Perkin Elmer Cetus). The forward primer was 5′-GAGGGAGGAGGGGGCTATGGGT-3′ and the reverse primer was 5′-TCCCTGGGCTGGGACTACTGG-3′. These primers generate a product of 271 bp which was cut into fragments of 182 + 89 bp in the presence of the *Rsa*I cutting site (G−60 allele).[18] The amplification protocol was: (1) one cycle of denaturation at 94°C for 3 min, annealing at 65°C and extension at 72°C; (2) 40 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 45 s, and (3) one final elongation cycle at 72°C for 10 min. A negative control without DNA was performed in every amplification run.

After amplification, PCR products were digested overnight at 37°C after adding 4 U of the restriction enzyme *Rsa*I to the PCR mixture. Resulting fragments were separated by electrophoresis in 2% agarose gels. Each gel was run for 2 h at 150 V, stained with ethidium bromide and photographed under UV transmitted light. The ΦX174 DNA digested with *Hae*III was used as length marker to estimate the size of the digested DNA fragments. The allele without the *Rsa*I restriction site is designated here as C−60 allele (271 bp), whereas the allele with the *Rsa*I restriction site is the G−60 allele (182 bp + 89 bp).

PCR amplification of the D19S178, CA/GT and D19S903 microsatellites. PCR amplifications of the three microsatellites were carried out in a volume of 10 μl containing 250 ng DNA, 125 μM each of dATP, dCTP, dGTP and dTTP, 10% buffer (100 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin, pH = 8.3 at 25°C), 0.5 pmol of the forward primer and 0.4 pmol of the reverse primer for the D19S178, 1.8 pmol of the forward primer and 5 pmol of the reverse primer for the CA/GT, 4 pmol of the forward and reverse primers for the D19S903 microsatellites, and 0.3 U of *Taq* polymerase (Perkin Elmer Cetus). For the D19S178 microsatellite, the forward primer was 5′-CACAACACTGTTCATTTGTC-3′ and the reverse primer was 5′-TTTCAGTAGAATTTCAGGCC-3′. The amplification protocol was: (1) one cycle of denaturation at 93°C for 5 min; (2) 30 cycles of denaturation at 94°C for 20 s; (3) annealing at 55°C for 40 s; and (4) extension at 72°C for 20 s. For the CA/GT,[10] the forward primer was 5′-AAACTGCACCTAATCTTCCC-3′ and the reverse primer was 5′-AGGCTGTGTTTCCCCAGACT-3′. The amplification protocol was: (1) one cycle of denaturation at 93°C for 5 min; (2) one cycle of annealing at 52°C for 1 min; and (3) 32 cycles of denaturation at 94°C for 20 s and annealing at 52°C for 60 s. For the D19S903 microsatellite, the forward primer was 5′-ACCGCACTCCACCCTG-3′ with an M13 sequence at the 5′ end (5′-CACGACGTTGTAAAACGAC-3′), and the reverse primer was 5′-TCCTCCTGTGAGATCCTCG-3′. The amplification protocol was: (1) one cycle of denaturation at 93°C for 5 min; (2) one cycle of annealing at 62°C for 1 min; (3) 10 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 1 min, and extension at 72°C for 5 s; and (4) 24 cycles of denaturation at 94°C for 20 s, annealing at 52°C for 1 min, and extension at 72°C for 5 s. Forward primers of the D19S178 and AC/GT microsatellites were labeled with an infrared tag (IRD800) (Li-Cor, Lincoln, NE, USA). An M13 sequence (5′-GTCGTTTTACAACGTCGTG-3′) tagged with IRD800 at a concentration of 5 pmol was used to label the forward primer of the D19S903 microsatellite.

After amplification, 1 μl of each PCR product was loaded on a 6% acrylamide urea gel on a DNA automatic sequencer (model 4000, Li-Cor, Lincoln, NE, USA). The genotyping was performed with a computer software (ONE-Dscan, Scanalytics, Billirica, MA, USA). Mendelian inheritance was confirmed within families for all markers typed on all subjects.

Statistical analysis

All statistical analyses were performed using SAS[24] and SAGE[25] software packages. The distributions of each variable were tested for normality using the Shapiro–Wilk *W*-test and those not normally distributed (FFAR, FFA50, FFA60% and INS) were $\log_e$ transformed. Age and gender differences for all variables were tested using ANOVA, while race differences were tested using ANOVA after adjusting for age effects. A chi-square test was performed to test for potential race differences in allele frequencies of all genetic markers. For microsatellites, alleles were pooled for the χ^2 tests when the expected value was less than 5. We also calculated the polymorphic information content (PIC).[26]

Linkage analysis. Phenotypes were adjusted for the effect of age (age, age^2, age^3) in each of the six gender-by-age (< 30, ≥ 30 – < 50 and ≥ 50-y-old) groups in white subjects and in each of the four gender-by-age (< 35 and ≥ 35-y-old) groups in black subjects using a stepwise regression, keeping only terms significant at the 5% level. ASF, AVF, ATF, FFAR, FFA50 and FFA60% were further adjusted for fat mass. For adjustment purposes, outliers (defined as those beyond ± 3 standard deviations (s.d.) from the mean of each gender and

age group's specific distribution) were temporarily set aside. The regression parameters were then used to calculate the residuals for all subjects including the outliers. Residuals were then standardized to a mean = 0 and s.d. = 1. The sib-pair linkage method implemented in the SIBPAL version 3.1 software from SAGE[25] was used to test for linkage between the phenotypes and the different markers.

Association. Two genotype groups were considered, ie carriers (genotypes C−60G and G−60G) and non-carriers (genotype C−60C) of the −60G allele. Association between the C−60G polymorphism and each phenotype was investigated separately in men and women using an ANCOVA (general linear model) procedure that included age (age, age^2, age^3) and race as covariates. ASF, AVF, ATF, FFAR, FFA50 and FFA60% were further adjusted for fat mass. Race-by-genotype interaction was estimated in the same model. In the presence of a significant gene-by-race interaction effect, the analysis was repeated in each race group separately. Least square means results obtained with the ANCOVA procedure for FFAR, FFA50 and FFA60% were back-transformed.

Results

Descriptive statistics for body fat phenotypes, plasma FFA and INS levels are summarized in Tables 1 and 2 for white and black subjects, respectively. Subjects of both races and genders were, on average, overweight with mean BMI values greater than 25 kg/m^2. In white subjects, all body fatness variables increased significantly across the three age groups in both genders. FFAR increased significantly with age in men but not in women. In black men and women, BMI, %FAT, AVF, ATF and FFAR increased significantly across the two age groups. White men (107 ± 3 cm^2) have higher AVF than black men (80 ± 5 cm^2, $P = 0.0001$). However, as compared to white women, black women exhibited a greater BMI (28.2 ± 0.4 *vs* 25.1 ± 0.3 kg/m^2; $P = 0.0001$), FAT (28.3 ± 0.9 *vs* 21.2 ± 0.7 kg; $P = 0.0001$), %FAT (36.5 ± 0.7 *vs* 30.2 ± 0.5%; $P = 0.0001$), SF8 (179 ± 5 *vs* 165 ± 3 mm; $P = 0.01$), ASF (350 ± 11 *vs* 293 ± 9 cm^2; $P = 0.0001$) and ATF (424 ± 13 *vs* 370 ± 11 cm^2; $P = 0.002$; results not shown). In both races, INS was not significantly different across age or gender groups (Table 1). However, black women exhibited a significantly greater INS (84.0 ± 3.9 pmol/l) than white women (59.9 ± 11 pmol l; $P = 0.0001$; results not shown).

Table 2 Body composition, plasma free fatty acid concentrations and fasting insulin in black subjects of the HERITAGE Family Study

	Age group (y)			
	Men		*Women*	
	< 35	*≥ 35*	*< 35*	*≥ 35*
Number of subjects	44–53	31–38	84–105	52–80
Age (y)	25.9 ± 0.7	46.7 ± 1.4	24.6 ± 0.5	44.1 ± 0.8
BMI (kg/m^2)	26.3 ± 0.7	28.4 ± 0.8^{1}	26.8 ± 0.6	30.0 ± 0.6^{2}
FAT (kg)	19.4 ± 1.6	23.3 ± 1.6	25.7 ± 1.4^{b}	31.5 ± 1.4^{c,1}
FATFR (kg)	65.2 ± 1.4	63.2 ± 1.4	46.1 ± 0.6^{c}	46.5 ± 0.8^{c}
%FAT (%)	21.4 ± 1.2	26.1 ± 0.9^{2}	33.9 ± 0.9^{c}	39.5 ± 0.9^{c,3}
SF8 (mm)	115 ± 9	132 ± 9	156 ± 6^{c}	213 ± 7^{c,3}
AVF (cm^2)	56 ± 5	112 ± 11^{3}	52 ± 3	94 ± 5^{3}
ASF (cm^2)	209 ± 24	261 ± 23	311 ± 19^{b}	398 ± 17^{c,2}
ATF (cm^2)	266 ± 28	373 ± 31^{1}	363 ± 21^{a}	491 ± 20^{b,3}
FFAR (μmol/l)	310 ± 34	432 ± 43^{1}	388 ± 31	472 ± 39^{1}
FFA50 (μmol/l)	213 ± 25	275 ± 29^{1}	272 ± 25	327 ± 28^{1}
FFA60% (μmol/l)	180 ± 20	275 ± 28^{2}	276 ± 23	346 ± 28^{1}
INS (pmol/l)	75.8 ± 8.6	73.2 ± 6.7	79.0 ± 6.6	85.8 ± 8.5

Mean ± s.e.m. Gender differences using ANOVA: $^{a}P < 0.05$; $^{b}P < 0.005$; $^{c}P < 0.0005$. Age group differences within each gender using ANOVA: $^{1}P < 0.05$; $^{2}P < 0.005$; $^{3}P < 0.0005$.

Table 1 Body composition, plasma free fatty acid concentrations and fasting insulin levels in white subjects of the HERITAGE Family Study

	Age group (y)					
	Men			*Woman*		
	< 30	*≥ 30 – < 50*	*≥ 50*	*< 30*	*≥ 30 – < 50*	*≥ 50*
Number of subjects	105–111	57–66	60–68	115–126	68–77	44–55
Age (y)	22.6 ± 0.3	40.1 ± 0.9	56.1 ± 0.5	22.4 ± 0.3	41.2 ± 0.8	55.3 ± 0.5
BMI (kg/m^2)	24.8 ± 0.4	28.6 ± 0.6	28.0 ± 0.5^{3}	23.1 ± 0.4^{a}	26.1 ± 0.5^{b}	27.9 ± 0.6^{3}
FAT (kg)	15.0 ± 1.0	24.6 ± 1.2	24.3 ± 1.1^{3}	16.8 ± 0.8	23.4 ± 1.2	28.6 ± 1.3^{a,3}
FATFR (kg)	63.6 ± 0.8	65.7 ± 0.9	61.5 ± 0.9^{1}	45.7 ± 0.5^{c}	46.2 ± 0.6^{c}	43.9 ± 0.7^{c,1}
%FAT (%)	17.8 ± 0.9	26.5 ± 0.9	27.6 ± 0.8^{3}	25.4 ± 0.8^{c}	32.4 ± 1.0^{c}	38.6 ± 0.9^{c,3}
SF8 (mm)	110 ± 5	155 ± 7	144 ± 6^{3}	144 ± 5^{c}	177 ± 6^{a}	201 ± 8^{c,3}
AVF (cm^2)	68 ± 4	127 ± 7	162 ± 8^{3}	45 ± 2^{c}	90 ± 5^{c}	129 ± 9^{b,3}
ASF (cm^2)	179 ± 13	282 ± 16	265 ± 12^{3}	234 ± 13^{b}	321 ± 15	379 ± 15^{c,3}
ATF (cm^2)	248 ± 17	410 ± 20	427 ± 18^{3}	279 ± 14	412 ± 19	508 ± 21^{b,3}
FFAR (μmol/l)	280 ± 23	348 ± 29	370 ± 30^{2}	368 ± 31	401 ± 40	467 ± 47
FFA50 (μmol/l)	193 ± 17	241 ± 20	238 ± 19^{2}	270 ± 25	286 ± 30	351 ± 34
FFA60% (μmol/l)	168 ± 15	223 ± 22	253 ± 21^{3}	307 ± 27^{c}	313 ± 28	382 ± 36
INS (pmol/l)	67.9 ± 4.2	65.3 ± 3.8	86.6 ± 9.1	60.5 ± 2.5	58.0 ± 3.1	63.5 ± 3.9

Mean ± s.e.m. Gender differences using ANOVA: $^{a}P < 0.05$; $^{b}P < 0.005$; $^{c}P < 0.0005$. Age group differences within each gender using ANOVA: $^{1}P < 0.05$; $^{2}P < 0.005$; $^{3}P < 0.0005$.

Allele and genotype frequencies for the C−60G polymorphism are presented in Table 3. Allele ($\chi^2=1.58$, d.f. = 1, $P>0.05$) and genotype ($\chi^2=2.08$, d.f. = 1, $P>0.05$) frequencies were not significantly different between races.

The allele frequencies were different between the two races for the D19S178 ($\chi^2=39.8$, d.f. = 10, $P<0.001$), CA/GT ($\chi^2=99.9$, d.f. = 8, $P<0.001$) and D19S903 ($\chi^2=39.7$, d.f. = 11, $P<0.001$) microsatellites and consequently the linkage analyses were performed separately in each race (data not shown). No significant linkage was observed between these markers and body fat traits in black subjects. However, in white subjects, weak linkages were observed between BMI and C−60G polymorphism ($P=0.04$, $n=318$), ASF and D19S903 ($P=0.01$, $n=311$), and between FFAR and FFA50 and D19S178 ($P=0.02$, $n=286$; $P=0.04$, $n=271$, respectively). Evidence of linkage was observed between ATF and D19S903 ($P=0.02$, $n=311$), FFAR and CA/GT and D19S903 ($P=0.006$, $n=291$; $P=0.0004$, $n=294$, respectively), FFA50 and CA/GT and D19S903 ($P=0.01$, $n=276$; $P=0.0003$, $n=282$, respectively) and FFA60% and CA/GT and D19S903 ($P=0.002$, $n=282$; $P=0.00005$, $n=288$, respectively).

The results of the association studies between the C-60G polymorphism of the LIPE gene and obesity-related phenotypes are presented in Table 4 for men and Tables 5 and 6 for women. In men, no evidence of gene-by-race interaction was

Table 3 Genotype and allele frequencies of the hormone-sensitive lipase C−60G polymorphism in biologically unrelated sedentary white and black parents of the HERITAGE Family Study

			Allele frequency[a]		*Genotype frequencies*		
	n	*PIC*	*C−60*	*G−60*	*C−60C*	*C−60G*	*G−60G*
White subjects	190	0.12	0.93	0.07	0.86	0.14	0
Black subjects	77	0.07	0.96	0.04	0.92	0.08	0

PIC, polymorphism information content.
[a]χ^2 for race difference in allele frequencies = 1.58, d.f. = 1, $P>0.05$.

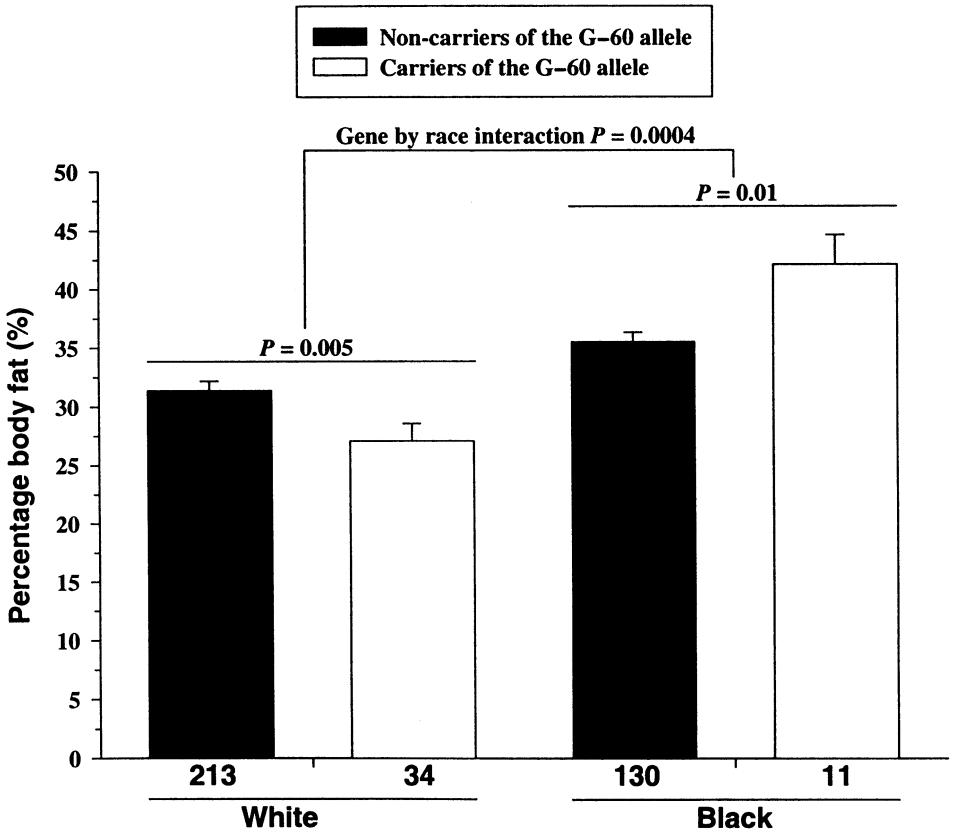

Figure 1 Association between the C−60G polymorphism and percentage body fat measured in white and black women. Data are means ± s.e.m. *P*-values were obtained using ANCOVA with age as covariate.

found and no differences were found between carriers and non-carriers for any of the phenotypes (Table 4). Table 5 presents the results for women. Carriers of the G allele exhibited a 5% higher FATFR than non-carriers. By contrast, strong race-by-gene interactions were observed for BMI ($P=0.0005$), FAT ($P=0.0007$), %FAT ($P=0.0003$, Figure 1), SF8 ($P=0.0001$), ASF ($P=0.03$) and ATF ($P=0.01$). Therefore, the associations between the C−60G polymorphism and these phenotypes were investigated separately in white and black women. Results presented in Table 6, indicate that white women carrying the G allele exhibited a significant lower %FAT (−14%, $P=0.005$) and SF8 (−15%, $P=0.01$) than non-carriers. However, in black women, the same allele was associated with a greater BMI (+19%, $P=0.004$), FAT (+38%, $P=0.009$), %FAT (+18%, $P=0.01$) and SF8 (+35%, $P=0.0009$). In women of both races, the C−60G polymorphism was not associated with abdominal obesity adjusted for FAT. The variance in adiposity phenotypes accounted for by the C−60G polymorphism ranged from 3.9 to 6.3% in white women and from 2.2 to 4.6% in black women.

To verify whether the associations observed between the C−60G polymorphism and body fat in women were influenced by insulin levels, the analyses were repeated with fasting insulin as a covariate in our models (results not shown). The results remained essentially the same in white women, but the association observed in black women for the adiposity variables (BMI, FAT, %FAT and SF8) became non-significant.

Table 4 Associations of the LIPE C−60G polymorphism with body composition and plasma free-fatty acid concentrations measured in men of both races

	n	*Non-carriers of the G−60 allele*	n	*Carriers of the G−60 allele*	P-value
BMI (kg/m^2)	289	27.0±0.3	43	26.7±0.7	0.72
FAT (kg)	274	20.8±0.7	41	20.2±1.6	0.73
%FAT (%)	274	23.5±0.5	41	22.3±1.2	0.34
FATFR (kg)	274	63.6±0.5	41	64.2±1.3	0.61
SF8 (mm)	262	130±4	38	123±8	0.43
AVF (cm^2)	274	97±2	40	94±6	0.63
ASF (cm^2)	273	227±3	40	222±7	0.43
ATF (cm^2)	274	324±3	40	316±8	0.32
FFAR (μmol/l)	263	249±15	40	190±29	0.06
FFA50 (μmol/l)	258	166±10	40	141±19	0.25
FFA60% (μmol/l)	259	150±9.4	39	125±19	0.27

Data are least square mean±s.e.m. (see text for details).

Table 5 Associations of the LIPE C−60G polymorphism with body composition and plasma free-fatty acid concentrations measured in women of both races

	n	*Non-carriers of the G−60 allele*	n	*Carriers of the G−60 allele*	P-value
FATFR (kg)	343	45.4±0.4	45	47.7±0.8	0.007
AVF (cm^2)	337	71±2	43	68.4±4.7	0.54
FFAR (μmol/l)	316	250±21	42	233±44	0.74
FFA50 (μmol/l)	303	183±16	41	166±31	0.58
FFA60% (μmol/l)	315	206±16	41	190±34	0.66

Data are least square mean±s.e.m. (see text for details).

Discussion

The present report shows that a mutation in the promoter region of the LIPE gene is associated with adiposity in women. It has been suggested that the DNA sequence between nucleotides −86 and −57 is necessary for the expression of HSL.[27] Moreover, the C−60G polymorphism has been shown to affect the expression of HSL. *In vitro* studies showed that the genomic sequence (−86 to +85 bp upstream of the transcription start site) with the −60G nucleotide inserted into a pGL3Enhancer vector resulted in a 38.5% lower luciferase activity compared to the wide-type −60C construct.[18] The C−60G polymorphism is in close proximity of a sterol response element (SRE-1),[18] which is a good candidate for explaining the gender difference in associations we observed. However, since HSL activity was not measured in the present study, further studies are needed to verify whether carriers of the G allele have lower HSL activity or gene expression compared to non-carriers.

Reports dealing with the association between HSL expression and obesity or regional fat distribution phenotypes have yielded inconsistent results. A recent *in vitro* study suggested that HSL expression (mRNA and protein levels) was diminished in subcutaneous fat depots of obese subjects.[8] In

Table 6 Associations of the LIPE C−60G polymorphism with obesity-related phenotypes measured in white and black women

	White women					*Black women*				
	n	*Non-carriers of the G−60 allele*	n	*Carriers of the G−60 allele*	P-value	n	*Non-carriers of the G−60 allele*	n	*Carriers of the G−60 allele*	P-value
BMI (kg/m^2)	223	25.4±0.4	34	24.1±0.8	0.13	169	27.9±0.5	12	33.1±1.7	0.004
FAT (kg)	213	22.2±1.0	34	19.2±1.8	0.10	130	26.7±1.2	11	36.8±3.6	0.009
%FAT (%)	213	31.4±0.8	34	27.1±1.5	0.005	130	35.5±0.8	11	42.1±2.5	0.01
SF8 (mm)	210	171±5	31	146±10	0.01	125	175±6	10	236±17	0.0009
ASF (cm^2)	211	282±5	32	267±10	0.13	126	345±6	11	365±17	0.25
ATF (cm^2)	211	358±6	32	338±11	0.06	126	409±6	11	443±19	0.08

Data are least square mean±s.e.m. (see text for details).

another report,[6] the ability of catecholamines to stimulate lipolysis was lower in abdominal subcutaneous fat depots of normal-weight subjects with a family history of obesity but HSL mRNA levels did not differ. Fat depot differences have been observed for the HSL mRNA levels, with higher levels in abdominal subcutaneous than in abdominal visceral adipocytes both in lean and in obese subjects.[28] In another report, however, no differences were observed.[29] Thus, further studies are needed to clarify whether HSL mRNA expression varies among fat depots or between normal-weight and obese subjects. In addition to its expression in adipose tissue, hormone-sensitive lipase was shown to be expressed in the skeletal muscle and is under the control of β-adrenergic receptors.[30] The association we observed between the C-60G polymorphism and fat-free mass suggests that the mobilization of triglycerides stored in the cytoplasm of myocites is less important in women carriers of the -60G allele independent of the race.

In addition to regional variation and significant correlations with fat cell size,[28] HSL activity is reduced in subcutaneous fat depots of obese subjects independent of gender[8] and in normal-weight subjects with a family history of obesity.[6] In obese subjects, the activity of HSL may be an important factor determining whether abdominal subcutaneous fat cells have low or high lipolytic capacity independent of the effect of fat cell size.[7]

The results of the present study suggest that the effects of the LIPE gene marker on obesity-related phenotypes are gender- and race-specific. Thus, our results indicated no association with adiposity in men, while strong evidence of association was observed in women but in opposite directions in black compared to white subjects, particularly for percentage body fat (see Figure 1) and sum of SF8. Previous studies suggested that adipocytes from abdominal subcutaneous fat depots of obese subjects are characterized by a reduced HSL activity.[8] Moreover, we have shown that black women have more subcutaneous fat than white women. We can thus speculate that black women carriers of the G allele have a reduced HSL activity in the subcutaneous fat depots, leading to a greater fat accumulation (lower fat mobilization) than non-carriers and resulting in high body fat. Since association between the C$-$60G polymorphism and body fat is race specific, other unknown factors may influence the effect of the G allele on body fat in white women. In the present study, a trend was observed in men for an association between the C$-$60G polymorphism and FFAR and linkages were observed between LIPE markers and FFAR, FFA50 and FFA60%. Thus, these results suggest that the LIPE gene or a gene in linkage disequilibrium with the LIPE gene plays a role in determining FFA levels.

The HSL pathway is regulated by a number of hormones, including insulin, which decreases its activity by reducing phosphorylation.[6,8] HSL activity could be related to the development of insulin resistance since continuously elevated FFA levels might play a role in the pathogenesis of type 2 diabetes in predisposed individuals by impairing peripheral glucose utilization and promoting hepatic glucose overproduction.[31] Two recent association studies showed some evidence that LIPE could represent a susceptibility gene for obesity and type 2 diabetes.[13,16] As proposed by Jiang *et al*,[32] black adolescent women have greater levels of insulin than their white counterparts, suggesting that they are more prone to develop insulin resistance. This result was also observed in the HERITAGE cohort with adult black women exhibiting greater levels of insulin than white women. These results suggest that a greater level of insulin resistance status observed in black women will lead to a lower inhibition of the hormone-sensitive lipase activity and thus aggravation of the pathogenesis of type 2 diabetes. The associations that were observed in the present study between the C$-$60G polymorphism and BMI, fat mass, FAT% and sum of SF8 in black women became nonsignificant after adjustment for fasting insulin, suggesting that the effects of the C$-$60G polymorphism on body fat are strongly influenced by insulin levels. Thus, the greater amount of subcutaneous fat and insulin levels found in black women compared to white women may account for the disappearance of the association after adjustment for insulin.

In summary, we observed strong gene-by-race interactions between the C$-$60G polymorphism in the LIPE gene and adiposity phenotypes in women only, but the strength of the interaction observed depends on fasting insulin levels. The C$-$60G polymorphism was associated with higher adiposity in black women, while it was associated with a reduced amount of fat in white women. In black women, the association disappeared when fasting insulin was taken into account. We suggest that the LIPE gene may play a role in variation in adiposity and that its effect is gender-, race- and insulin-dependent.

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