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Associations between the Leptin Receptor Gene and Adiposity in Middle-Aged Caucasian Males from the HERITAGE Family Study*

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ABSTRACT

Linkage and association studies between three exonic polymorphisms in the leptin receptor gene and body composition variables in the HERITAGE Family Study were undertaken. Polymorphisms K109R, Q223R, and K656N have been analyzed with body mass index (BMI), sum of height skinfolds (SF8), fat mass (FM), percent body fat (%FAT), fat free mass, and plasma leptin level. Single-point linkage analysis and covariance analysis across genotypes were performed, by race, on phenotypes adjusted for age and sex. Blacks (88 parents; 231 adult offspring) from 115 nuclear families (72–119 sibpairs) and Caucasians (192 parents; 330 adult offspring) from 99 nuclear families (319–364 sibpairs) were used for these analyses. In Caucasians, BMI and FM showed suggestive linkages with K109R ($P = 0.02$ and $P = 0.05$, respectively) and associations with Q223R ($P = 0.005$ and $P = 0.03$, respectively). In blacks, no statistically significant linkage or association was observed. In Caucasians, associations with Q223R were observed in parents, but not in offspring, for BMI, FM, and %FAT ($0.04 \leq P \leq 0.0001$). Males, not females, showed differences across genotypes for the same phenotypes plus SF8 and leptin ($0.03 \leq P \leq 0.0002$). Carriers of the R223 allele showed higher values than noncarriers for BMI (+4 U, $P = 0.0001$), SF8 (+30 mm, $P = 0.01$), FM (+7 kg, $P = 0.0004$), %FAT (+5%, $P = 0.0002$), and leptin (+4 ng/mL, $P = 0.0006$). These results indicate a significant effect of leptin receptor on adiposity in middle-aged Caucasian males. (*J Clin Endocrinol Metab* **85:** 29–34, 2000)

RODENT LEPTIN (LEP) and leptin receptor (LEPR) gene products have defined a new biological pathway for the regulation of food intake and energy expenditure. Leptin is released from adipocytes as a signal of body fat stores and acts as a satiety factor with its receptor located mainly in the hypothalamus, a brain area known to be involved in the regulation of food intake. The LEP and LEPR genes have been cloned in humans (1, 2),and mapped to 7q31.3 (3) and 1p31 (4, 5), respectively. In recent studies, two mutations in LEP (6, 7) and one in LEPR (8) have been shown to produce severe early-onset obesity, with concomitant perturbations of different hormonal and physiological processes. Similarly, mutations producing severe obesity in humans were also reported in three other genes: the prohormone convertase one (9), the POMC (10), and the melanocortin receptor 4 (11, 12). However, all these single-gene mutations explain few obesity cases, and the causes of the genetic predisposition for the majority of the human obesity cases remain unexplained.

No linkages were observed between markers in the vicinity of *LEPR* and adiposity in Pima Indians (13, 14) and in French subjects (15), whereas positive linkages were observed in the Québec Family Study (16). On the other hand, some 19 polymorphisms have been reported in the human LEPR among the 20 different exons and introns of the gene (15, 17–24). The potential effects of these polymorphisms have been evaluated in different populations, with few positive results. In Pima Indians, allele frequencies were shown to be different between 10 lean and 10 obese subjects for 2 intronic and 1 exonic nucleotide changes ($P = 0.003$) and for

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haplotypes of the same 3 allelic variants and a Q223R substitution ($P = 0.001$) (20). Association was also reported between a pentanucleotide insertion/deletion polymorphism in the 3′untranslated region of LEPR and insulin levels in obese subjects, particularly in females, in the fasted state ($P = 0.0004$) or after an oral glucose tolerance test ($P = 0.02$) (15, 24). In both cases, carriers of the insertion allele showed lower insulin values. In the Québec Family Study, linkages ($0.004 \leq P \leq 0.02$) were observed between LEPR and different adiposity and body composition variables, the strongest results being observed between Q223R and fat mass (FM; $P = 0.004$) and between a CTTT repeat in intron 16 and fat free mass (FFM; $P = 0.006$) (25). In the latter study, carriers of the Q223 allele had 4 kg less FFM ($P = 0.005$) in males with a body mass index (BMI) < 27 kg/m^2. The same was true for carriers of the less frequent allele at a CTTT repeat ($P = 0.005$) in women with a BMI ≥ 27 kg/m^2 (25). On the other hand, negative results were reported for Q223R and for other polymorphisms in American (17, 26); British (21), except for a weak association between a K656N substitution and BMI in lean (BMI < 22 kg/m^2) male subjects ($P = 0.02$); Danish (22); Japanese (23); and French (15) populations.

We have analyzed three polymorphisms (K109R, Q223R, and K656N) located in exons 4, 6, and 14 of LEPR to test for linkage and association with adiposity and body composition variables in the HERITAGE Family Study. The HERITAGE cohort includes Caucasian subjects, but also blacks who have not been investigated yet for variation in LEPR.

Subjects and Methods

Subjects and phenotypes

The HERITAGE Family Study cohort has been previously described (27). HERITAGE included nonascertained (according to obesity) black and Caucasian nuclear families from the greater Québec City; Phoenix, AZ; Minneapolis, MN; Austin, TX; and Indianapolis, IN areas. Subjects were tested for a battery of morphometric and physiological variables before and after a 20-week exercise program. The study protocol had been previously approved by the Human Subjects Committee at each participating institution. Informed written consent was obtained from each subject. Only baseline data, *i.e.* before the exercise program, were used for the present study. Blood samples were obtained for various biochemical assays, and permanent lymphoblastoid cell lines were established for the extraction of DNA. A total of 319 black subjects (88 parents and 231 offspring) from 115 families and 522 Caucasians (192 parents and 330 offspring) from 99 families were available for the study. Dependent variables include BMI (weight in kg divided by height in m^2) and percent body fat (%FAT) estimated from body density measurements obtained by underwater weighing and the equations of Siri (28) and Lohman (30) for Caucasian men and women, respectively, and of Schutte (31) and Ortiz (32) for black men or women, respectively. FM (kg) and FFM (kg) were calculated from %FAT body fat and body weight. Pulmonary residual volume was assessed by the helium dilution technique (33) or oxygen dilution (34, 35) techniques. Subcutaneous fat (mm) was estimated by the sum of height skinfold thicknesses (SF8 = abdominal, subscapular, suprailiac, medial calf, triceps biceps, midaxillary, and thigh). Leptin level (ng/mL) was evaluated by an RIA (Linco Research, Inc., St. Charles, MO) in which the lowest quantity detectable was 0.5 ng/mL in plasma.

Molecular analysis

Genomic DNA was prepared from permanent lymphoblastoid cells by the proteinase K and phenol/chloroform technique. DNA was dialyzed four times against TE buffer (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) for 6 h at 4 C, and ethanol was precipitated. The three restriction fragment length polymorphisms analyzed have been described elsewhere (19). PCR was performed on a Perkin-Elmer Corp. 9600 apparatus using 100 or 200 ng genomic DNA, 300 nmol/L of each primer, 200 μmol/L deoxynucleotides, and 0.5 U *Taq* polymerase in PCR buffer (Roche Molecular Biochemicals, Laval, SC) with 1.5 mmol/L $MgCl_2$, for a final vol of 10 μL. PCR cycles consisted of 40 cycles at 94 C for 30 sec, annealing at 55 C for 30 sec, and extension at 72 C for 30 sec, with a final extension of 10 min at 72 C. PCR products were digested for 12 h at 37 C with 5 U *Hae*III, 5 U *Msp*I, or 5 U Bst UI restriction enzymes, an isoschizomer of the Mvn I enzyme used originally (19), for the K109R, Q223R, and K656N polymorphisms, respectively. The resulting fragments were separated on 2.5–3% agarose gels.

Statistical analysis

Phenotypic variables were adjusted, within race, sex and age groups, with the latter defined as lower then 35, between 35 and 50, and more than 50 yr old, for age, age^2, and age^3 using a regression procedure in which outliers (± 3 SD) were excluded for the estimation of the regression parameters. Residuals from all subjects, including outliers, were then standardized to a mean of 0 and an SD of 1. The sibpair linkage analysis was performed on nuclear families using the SIBPAL version 3.0 software from S.A.G.E. (Statistical Analysis for Genetic Epidemiology) (36) with the population allele frequencies estimated, by race, from unrelated subjects. Association studies were undertaken on all subjects from both generations, because pooling all family members in each race produced unbiased residuals, even in the presence of extremely correlated clusters (Province MA, Rice T, Rao PC, unpublished data). Phenotypes were compared between genotypes using covariance analysis with the same covariates as for linkage analysis plus clinical center of origin. A chi-square test was used to compare allele frequencies and genotype distributions between black and Caucasian subjects and to test for Hardy-Weinberg equilibrium of the genotype distribution of the polymorphisms. Statistical Analysis System (version 6.08) for PC was used for the analysis.

TABLE 1. Allele frequencies ($\pm$SE) for three exonic polymorphisms in the LEPR gene in the HERITAGE Family Study, the Québec Family Study, and in two other populations

Polymorphisms	Allele	Heritage		Québec[a]	Japanese[b]	Pima[c]
		Blacks	Caucasians			
K109R	K	0.86 ± 0.02	0.73 ± 0.02	0.71 ± 0.02	0.79 ± 0.03	0.42 ± 0.08
	R	0.14 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.21 ± 0.03	0.58 ± 0.08
Q223R	Q	0.49 ± 0.05	0.54 ± 0.02	0.55 ± 0.02	0.15 ± 0.02	0.25 ± 0.07
	R	0.51 ± 0.05	0.46 ± 0.02	0.45 ± 0.02	0.85 ± 0.02	0.75 ± 0.07
K656N	K	0.83 ± 0.02	0.82 ± 0.01	0.77 ± 0.02	0.88 ± 0.02	NA
	N	0.17 ± 0.02	0.18 ± 0.01	0.23 ± 0.02	0.12 ± 0.02	NA

NA, Not available.
[a] Adapted from Chagnon *et al*. (25).
[b] Adapted from Matsuoka *et al*. (23).
[c] Adapted from Thompson *et al*. (20).

Results

The allele frequencies for blacks and Caucasians for the K109R, Q223R, and K656N polymorphisms in *LEPR*, and the haplotypes of these three polymorphisms, are presented in Table 1. A Hardy-Weinberg equilibrium is observed for the three polymorphisms and their haplotypes within both races. The allele frequencies for Caucasians in the HERITAGE Family Study are similar to those reported in the Québec Family Study, whereas blacks differ significantly from Caucasians for genotype and allele distributions at the K109R polymorphism (Table 2). The allele frequencies are comparable with those observed in British (21), Danish (22), American (26), and French (20) Caucasian populations. Both blacks and Caucasians from the HERITAGE Family Study, as well as Caucasians from other populations, exhibit differences for K109R with Pima Indians, and for Q223R with Japanese and Pima Indians (Table 1). Therefore, allelic variations in *LEPR* are characterized by a significant race component with black, Japanese, and Pima Indian populations, showing specific allelic frequency differences among them, whereas Caucasian subpopulations exhibit homogeneous frequencies. Similar differences in haplotype frequencies (Table 1B) are observed between blacks and Caucasians in HERITAGE, with four out of the six observed haplotypes showing significant differences ($0.04 \leq P \leq 0.001$). The three polymorphisms are also strongly in linkage disequilibrium in Caucasians ($\chi^2 = 166.38$; 7 df; $P < 0.0001$) and weakly in blacks ($\chi^2 = 18.07$; 7 df; $P < 0.02$). Taken two by two, K109R and Q223R showed the strongest disequilibrium in Caucasians ($\chi^2 = 114.04$; df = 3; $P < 0.001$), whereas no disequilibrium were observed in blacks ($P > 0.05$).

Descriptive statistics for the different phenotypic variables in the HERITAGE Family Study for blacks and Caucasians, within each of the four generations, by sex groups, are shown in Table 3. Mean BMI for unrelated subjects of both sexes from the parental generation was 28.8 kg/m^2 in blacks (n = 88; range, 19–43) and 28.0 kg/m^2 in Caucasians (n = 192; range, 19–48 kg/m^2). In parents, normal-weight (BMI $<$ 25 kg/m^2), overweight (25 $\leq$ BMI $<$ 30 kg/m^2), and obese (BMI $\geq$ 30 kg/m^2) subjects (38) are present in both blacks (22%, 43%, and 35%, respectively) and Caucasians (27%, 42%, and 31%, respectively), with no significant difference in the distribution between the two groups ($\chi^2 = 1.099$; 2 df; $P = 0.58$). Similarly, no significant differences in the leptin levels, adjusted for age and sex, were observed between black and Caucasian parents. Black children had a lower mean BMI, of 27.7 kg/m^2 (n = 231, range, 17–51), and a different BMI distribution (42%, 27%, and 32%, respectively) than their parents. Similarly, Caucasian children are leaner than their parents, with a mean BMI of 24.6 kg/m^2 (n = 330, range, 17–44 kg/m^2) and a BMI distribution of 63%, 24%, and 13%, respectively. Fifty seven percent (black males) to 74% (Caucasian males) of the leptin variance is explained by the FM of the subjects.

For the single-locus sibpair linkage analysis (Table 4), the number of sibpairs varied, depending on marker and phenotype, with a range from 75–119 pairs in blacks and 317–364 pairs in Caucasians. Suggestive linkages were observed in Caucasians for K109R with BMI ($P = 0.02$) and FM ($P = 0.05$), with borderline P values of 0.10 for SF8 and 0.11 for FFM. No statistically significant linkage was observed in blacks.

Evidence of association has been observed only in Caucasians and for the Q223R polymorphism with BMI ($P = 0.005$) and FM ($P = 0.03$) (Table 5). No evidence of association was observed in blacks, and in Caucasians for the K109R and K656N polymorphisms. Association analyses for Q223R

TABLE 2. Chi-Square test at three different exonic polymorphisms in the LEPR gene between Blacks and Caucasians in the HERITAGE Family Study

A, For individual genotypic and allelic variations

		Polymorphisms								
		K109R			Q223R			K656N		
		Blacks	Caucasians	*P*	Blacks	Caucasians	*P*	Blacks	Caucasians	*P*
GENOTYPES	1/1	0.73 (56)	0.53 (101)	0.004	0.23 (18)	0.32 (60)	0.41	0.68 (53)	0.66 (125)	0.84
	1/2	0.27 (21)	0.40 (76)		0.51 (39)	0.45 (87)		0.29 (22)	0.32 (61)	
	2/2	0.0 (0)	0.07 (13)		0.26 (20)	0.23 (43)		0.03 (2)	0.02 (4)	
ALLELES	1	0.86	0.73	0.001	0.49	0.54	0.23	0.83	0.82	0.73
	2	0.14	0.27		0.51	0.46		0.17	0.18	

Proportions and number of cases (in *parentheses*) are given for the genotypes, and proportions for the alleles. Wild-type (1) and variant (2) alleles.

B, For haplotypes of the three polymorphisms [number of subjects carrier of the haplotype by race, and the number observed for each haplotype by race (Total), are compared]

HAPLOTYPES			Blacks		Caucasians			Total		
109	223	656	Carriers	Noncarriers	Carriers	Noncarriers	*P*	Blacks	Caucasians	*P*
K	Q	K	0.69 (53)	0.31 (24)	0.57 (109)	0.43 (81)	0.08	0.43 (66)	0.36 (136)	0.001[a]
K	Q	N	0.10 (8)	0.90 (69)	0.68 (60)	0.32 (130)	0.001	0.05 (8)	0.18 (68)	
K	R	K	0.48 (37)	0.52 (40)	0.34 (65)	0.66 (125)	0.04	0.27 (41)	0.19 (73)	
K	R	N	0.22 (17)	0.78 (60)	0.005 (1)	0.995 (189)	0.001	0.12 (18)	0.003 (1)	
R	R	K	0.26 (20)	0.74 (57)	0.45 (86)	0.56 (104)	0.004	0.13 (20)	0.26 (99)	
R	Q	K	0.01 (1)	0.99 (76)	0.02 (3)	0.98 (187)	0.86	0.007 (1)	0.008 (3)	

Proportions and number of cases (in *parentheses*) are given. R109/Q223/N656 and R109/R223/N656 haplotypes have not been observed.
[a] R109/Q223/K656 have not been included in the chi-square test because of too low (<5.0) expected values.

TABLE 3. Number of subjects (N), mean and range by race, generation and sex for each phenotypic variable

Variables	Parent						Adult offspring					
	Male			Female			Male			Female		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Blacks												
Age (yr)	29	50.0	39–66	60	46.6	34–65	88	27.0	16–46	149	27.6	16–48
BMI (kg/m^2)	29	27.5	19–42	59	29.4	20–43	84	27.4	17–44	147	27.9	18–51
SF6 (mm)	24	96.1	37–193	44	168.3	51–251	78	91.6	27–200	113	133.8	51–267
FM (kg)	25	21.3	10–49	39	31.2	10–56	81	20.7	2–55	121	27.1	8–73
Leptin (ng/mL)	25	7.3	1–25	49	28.5	6–82	82	7.8	<1–50	125	27.5	3–104
Fat (%)	25	25.3	16–43	39	39.2	21–51	81	22.3	3–41	121	35.1	16–55
FFM (kg)	25	60.2	49–69	39	46.3	35–61	81	65.5	41–93	121	46.3	28–69
Caucasians												
Age (yr)	99	53.5	44–64	95	52.0	42–65	164	25.2	17–40	172	25.4	17–41
BMI (kg/m^2)	98	28.4	21–41	94	27.6	19–48	161	25.7	17–44	169	23.7	17–39
SF6 (mm)	88	113.5	44–273	82	159.8	70–291	156	92.5	31–208	168	118.9	40–261
FM (kg)	92	24.6	8–58	85	27.0	10–62	150	17.0	<1–53	169	18.1	3–59
Leptin (ng/mL)	97	9.0	2–30	88	24.0	5–70	155	5.8	1–30	165	15.2	2–82
Fat (%)	92	27.6	11–42	85	36.6	20–54	150	19.6	<1–43	169	26.7	7–53
FFM (kg)	92	62.3	47–80	85	44.5	35–57	150	64.2	45–87	169	46.1	34–65

were also performed in Caucasians for each generation and sex separately (Table 5). Only parents were characterized by significant associations with BMI, FM, and %FAT ($0.04 \leq P \leq 0.0001$). Moreover, in Caucasian parents, males (not females) showed differences for the same phenotypes, plus SF8 and leptin ($0.03 \leq P \leq 0.0002$). In these subjects, carriers of the R223 allele showed higher values than noncarriers for BMI (+4 units, $P = 0.0001$), SF8 (+30 mm, $P = 0.01$), FM (+7 kg, $P = 0.0004$), %FAT (+5%, $P = 0.0002$), and leptin (+4 ng/mL, $P = 0.0006$). Haplotype analysis (data not shown) yielded weak evidence of association in Caucasians only, with carriers of the K109/Q223/K656 haplotype showing a lower BMI (−1 unit, $P = 0.04$) and FM (−2 kg, $P = 0.04$), and carriers of the R109/R223/K656 haplotype a slightly higher BMI value (+1 unit, $P = 0.05$) than other haplotypes.

Discussion

Significant differences in allele and genotype frequencies for the K109R polymorphism and for haplotypes of the three exonic polymorphisms studied have been observed between black and Caucasian subjects in the HERITAGE Family Study cohort. This difference in allele frequencies does not seem to be related to adiposity or body composition, because this polymorphism did not show any linkage or association with these phenotypes in blacks. Similarly, in Pima Indians, who showed an even stronger difference in allele frequencies at K109R with Caucasian groups, no apparent relation with obesity was observed (20).

Here, we report weak evidence of linkages for K109R in Caucasians with BMI and FM (Table 4) but without association (data not shown). In contrast, no linkage was observed for Q223R, but strong associations were found (Table 5). We obtained the same kind of nonconsistent results in the Québec Family Study for Q223R, which showed strong positive linkages with adiposity phenotypes but negative associations (25). This is what is expected under linkage equilibrium. For instance, polymorphims K109R and Q223R are in strong linkage in Caucasians ($\chi^2 = 114.04$, 3df; $P < 0.001$) but not in blacks ($\chi^2 = 7.33$, 3 df; $P > 0.05$). Evidence for linkage does not depend on which allele is shared by sibs. In contrast, associations are detected by the apparent effects of a marker with such a functional mutation. Consequently, linkage can be observed with a specific locus without allelic association and vice-versa, particularly as here, when the two polymorphisms are in linkage disequilibrium. Moreover, association analysis is more sensitive than linkage analysis, and so association can be detected without linkage. The Q223R polymorphism exhibited evidence of an association in Caucasians only, and, more particularly, in parents (Table 5). Caucasian adult offspring are leaner than their parents, as reflected by mean BMI and BMI classes distribution. They are also younger, with no overlap in age range (Table 3b). Similar differences are observed in blacks (Table 3a). Differences in the association results between black and Caucasian parents could come from a race effect on LEPR expression, because biological characteristics, such as BMI and age, are similar. The difference between blacks and Caucasians for allele and haplotype distributions, and the presence (Caucasians) or absence (blacks) of linkage disequilibrium between the markers, support the hypothesis that two different gene pools have been sampled. These differences in LEPR expression could come from modulating or permissive factors acting on different genetic backgrounds, as has been described in rodent models of obesity (39, 40, 41). In Caucasians, associations were observed only in males, whereas younger and leaner subjects showed no effect of LEPR polymorphisms.

Negative results have been reported for the Q223R polymorphism in other Caucasian populations. In a study of American subjects, a comparison across genotypes was performed for obese subjects only, with males and females pooled together (26). Similarly, a negative association with BMI was reported in British males with a BMI < 28 kg/m^2 or with a BMI ≥ 28 kg/m^2, analyzed separately (21). In the present study, if Caucasian male parents are divided into the same two BMI categories (BMI < 28 kg/m^2 *vs.* BMI ≥ 28 kg/m^2), no associations with Q223R are observed with BMI ($P = 0.23$ and $P = 0.21$, respectively). Finally, in a Danish

TABLE 4. Sibpair linkage results between allelic variation in the gene and adiposity-related variables

Variables	K109R		Q223R		K656N		Haplotypes	
	N	*P*	N	*P*	N	*P*	N	*P*
Blacks								
BMI (kg/m^2)	115	0.53	119	0.68	119	0.57	119	0.69
SF8 (mm)	75	0.24	79	0.89	79	0.69	79	0.92
FM (kg)	89	0.58	92	0.54	92	0.36	92	0.68
Leptin (ng/mL)	91	0.36	94	0.32	94	0.70	94	0.50
Fat (%)	89	0.79	92	0.77	92	0.69	92	0.90
FFM (kg)	89	0.09	92	0.88	92	0.71	92	0.82
Caucasians								
BMI (kg/m^2)	358	**0.02**	358	0.13	358	0.74	353	0.18
SF8 (mm)	338	0.10	338	0.73	338	0.83	333	0.61
FM (kg)	338	**0.05**	338	0.22	338	0.76	333	0.35
Leptin (ng/ml)	336	0.25	336	0.36	336	0.47	331	0.34
Fat (%)	338	0.28	338	0.60	338	0.73	333	0.75
FFM (kg)	338	0.11	338	0.24	338	0.79	333	0.62

N, Number of sibpairs. Significant results at $P \leq 0.05$ are in *bold*.

TABLE 5. Covariance analysis in Caucasians of the body composition variables by generation and sex for the Q223R polymorphism in the LEPR gene

	Geno	Total				Generations								Parents							
						Parents				Children				Males				Females			
		N	Mean	SE	*P*	N	Mean	SE	*P*	N	Mean	SE	*P*	N	Mean	SE	*P*	N	Mean	SE	*P*
BMI (kg/m^2)	Q/Q	152	24.9	0.4	**0.005**	60	26.0	0.6	**0.0001**	92	24.0	0.5	0.38	31	25.8	0.7	**0.0002**	29	26.2	0.9	0.11
	Q/R	241	26.3	0.3		87	29.2	0.5		153	24.8	0.4		42	29.9	0.6		45	28.6	0.7	
	R/R	109	26.2	0.4		43	27.9	0.7		66	25.1	0.5		24	29.1	0.8		19	26.8	1.1	
SF8 (mm)	Q/Q	140	143.3	4.5	0.38	52	158.7	7.7	0.15	88	134.9	5.6	0.67	28	125.4	9.4	**0.03**	24	191.1	11.2	0.36
	Q/R	222	150.3	3.6		72	178.9	6.4		149	135.0	4.3		34	153.4	8.3		38	204.8	8.8	
	R/R	103	151.4	5.2		40	170.5	8.7		63	141.6	6.5		22	160.9	10.6		18	183.0	12.8	
FM (kg)	Q/Q	147	18.9	0.8	**0.03**	57	22.6	1.2	**0.003**	90	16.9	1.0	0.12	30	19.9	1.6	**0.002**	27	24.9	1.9	0.10
	Q/R	228	21.2	0.6		80	28.2	1.0		147	17.3	0.8		40	27.4	1.3		40	29.4	1.6	
	R/R	103	21.8	1.0		38	25.0	1.5		65	19.9	1.2		21	25.9	1.9		17	23.9	2.5	
% FAT	Q/Q	147	25.4	0.7	0.11	57	30.4	1.0	**0.04**	90	22.4	0.9	0.14	30	24.1	1.1	**0.0008**	27	36.6	1.4	0.07
	Q/R	228	27.2	0.6		80	33.5	0.8		147	23.6	0.7		40	29.7	0.9		40	38.1	1.2	
	R/R	103	27.1	0.8		38	30.6	1.2		65	25.2	1.1		21	28.5	1.3		17	32.8	1.8	
Leptin (ng/mL)	Q/Q	146	12.1	0.9	0.65	55	13.7	1.7	0.21	91	10.8	1.0	0.98	30	6.0	1.0	**0.002**	25	21.5	3.0	0.68
	Q/R	236	13.1	0.7		86	17.4	1.3		149	10.7	0.8		42	10.7	0.8		44	24.9	2.4	
	R/R	105	12.8	1.1		42	16.2	1.9		63	11.0	1.2		24	9.9	1.1		18	24.0	3.7	
FFM (kg)	Q/Q	147	53.3	0.7	0.20	57	51.4	1.1	0.06	90	55.0	0.8	0.11	30	60.6	1.4	0.31	27	42.3	0.9	**0.02**
	Q/R	228	54.2	0.6		80	54.9	1.0		147	53.6	0.6		40	63.0	1.2		40	45.8	0.8	
	R/R	103	55.3	0.9		38	54.7	1.4		65	55.8	0.9		21	63.5	1.7		17	44.5	1.2	

Mean and SE adjusted for age and sex (plus fat mass for leptin only). Geno, Genotypes; N, number of subjects. Significant results ($P \leq 0.05$) are in *bold*.

study, (22) homozygotes for Q223 allele were not included in the comparison of BMI across Q223R genotypes, and we observed here that the main differences are precisely between carriers of the R223 allele and the Q223Q homozygotes.

Two duplicate cytokine domains (C domain) have been reported in *LEPR* (2) that represent two putative leptin binding regions. The Q223R substitution is located in exon 6 within the first C domain. The Zucker rat mutation in the LEPR (*Lepr*^{fa}) is located in the first C domain of *LEPR* (19). *Lepr*^{fa} involves the substitution of a glutamine (Q) residue at position 269 (270 in humans) for a proline (P) residue, which affects the functionality of the receptor (42, 43, 44, 45). The Q223R polymorphism has been shown, in the present study, to be associated with adiposity variables such as BMI, SF8, FM, %FAT, and leptin. The Q223R polymorphism was also linked to BMI, SF6, and FM in the Québec Family Study (25). It could be hypothesized that the single amino acid change, a glutamine for an arginine, observed at the residue 223 in human exon 6, changes the signaling capacity of *LEPR*, as is observed for the *Lepr*^{fa} mutation in rat (42, 43, 44, 45). This single substitution effect could be comparable with the arginine to tryptophane substitution at codon 105 in the human LEP gene, which is sufficient to impair the normal processing of leptin through the secretory pathway (7).

Associations in the HERITAGE Family Study and linkages in the Québec Family Study were detected for the Q223R polymorphism but without reciprocal linkages in the present study or associations in the Québec Family Study. Differences in the proportion of lean, overweight, or obese subjects among the two groups are observed (27%, 42%, and 31%, respectively in HERITAGE *vs*. 48%, 27%, and 25%, respectively in the Québec Family Study). On the other hand, subjects from the Québec Family Study were exclusively of French descent, from the immediate region of Québec City, whereas Caucasians from the HERITAGE Family Study were

recruited in Québec City but also at three centers in the United States. When the two subgroups are analyzed separately, it can be seen that similar (BMI, $P = 0.004$ *vs.* 0.02; FM, $P = 0.07$ *vs.* 0.05) and divergent (SF8, $P = 0.52$ *vs.* 0.12; %FAT, $P = 0.46$ *vs.* 0.09; leptin, $P = 0.59$ *vs.* 0.07) results are observed between subjects from the United States and Québec. Fewer Caucasians are available from the Québec Clinical Center, compared with the United States Clinical Centers (~70 vs. 120) for these analyses, but higher mean values of the different phenotypes for the R223 allele carriers are noted in both subsamples of Caucasians (data not shown).

In conclusion, there is a significant effect of the Q223R LEPR polymorphism on adiposity in humans. The effect is observed among middle-aged male Caucasians only, with carriers of the R223 allele exhibiting higher mean adiposity values. The specific effect of the Q223R substitution on the functionality of the LEPR remains to be investigated.

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