A Sib-Pair Analysis Study of 15 Candidate Genes in French Families With Morbid Obesity

Indication for Linkage With Islet 1 Locus on Chromosome 5q

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As part of an ongoing search for susceptibility genes in obese families, we performed linkage analyses in 101 French families between qualitative and quantitative traits related to morbid obesity and polymorphisms located in or near 15 candidate genes whose products are involved in body weight regulation. These included cholecystokinin A and B receptors (CCK-AR and CCK-BR), glucagon-like peptide 1 receptor (GLP-1R), the LIM/homeodomain islet-1 gene (Isl-1), the caudal-type homeodomain 3 (CDX-3), the uncoupling protein 1 (UCP-1), the β_3-adrenoceptor (β_3-AR), the fatty acid–binding protein 2 (FABP-2), the hormone-sensitive lipase (HSL), the lipoprotein lipase (LPL), the apoprotein-C2 (apo-C2), the insulin receptor substrate-1 (IRS-1), the peroxisome proliferator-activated receptor-γ (PPAR-γ), tumor necrosis factor-α (TNF-α), and the liver carnitine palmitoyltransferase-1 (CPT-1). Phenotypes related to obesity such as BMI, adult life body weight gain, fasting leptin, insulin, fasting glycerol, and free fatty acids were used for nonparametric sib-pair analyses. A weak indication for linkage was obtained between the Isl-1 locus and obesity status defined by a *z* score over one SD of BMI (*n* = 226 sib pairs, π = 0.54 ± 0.02, *P* = 0.03). Moreover, a suggestive indication for linkage was found between the Isl-1 locus and BMI and leptin values (*P* = 0.001 and 0.0003, respectively) and leptin adjusted for BMI (*P* = 0.0001). Multipoint analyses for leptin trait with Isl-1 and two flanking markers (D5S418 and D5S407) showed that the logarithm of odds (LOD) score is 1.73, coinciding with the Isl-1 locus. Although marginally positive indications for linkage in subgroups of families were found with IRS-1, CPT-1, and HSL loci, our data suggested that these genes are not major contributors to obesity. Whether an obesity susceptibility gene (Isl-1 itself or another nearby gene) lies on chromosome 5q should be determined by further analyses. ***Diabetes* 48:398–402, 1999**

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β_3-AR, β_3-adrenoceptor; apo-C2, apoprotein-C2; CCK, cholecystokinin; CDX-3, caudal-type homeodomain 3 gene; CPT-1, carnitine palmitoyltransferase-1; FABP-2, fatty acid–binding protein 2; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; HSL, hormone-sensitive lipase; IRS-1, insulin receptor substrate-1; Isl-1, islet-1 gene; LOD, logarithm of odds; LPL, lipoprotein lipase; PPAR-γ, peroxisome proliferator-activated receptor-γ; UCP-1, uncoupling protein-1.

Human obesity is a multifactorial disease influenced by environmental and genetic factors (1). The identification of the genes involved in monogenic animal models for obesity have dramatically improved our knowledge of body weight regulation (2). Mutations in leptin (3), its receptor (4), and the proconvertase genes (5) have been found in rare monogenic forms of human obesity. Common human obesity cannot be readily reduced to simple Mendelian segregation patterns, but may result from a network of interactive genetic factors (1). Putative obesity loci were recently mapped in Pima Indians (6) and Mexican Americans (7), but few genetic loci have been identified in obese Caucasians (1,8,9). Therefore, we performed, in 101 morbidly obese French families, linkage analyses of 15 candidate genes whose products are involved in several processes.

First, we looked at genes whose products are involved in food intake regulation by the central nervous system, such as cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1). CCK, a satietogenic component in the hypothalamus, may regulate carbohydrate metabolism through an increase in insulin secretion. It mediates its activity through G-protein–coupled type A and B receptors (CCK-AR and CCK-BR) (10). GLP-1 is postulated to contribute to the regulation of both blood glucose metabolism and satiety (11), although GLP-1 disruption in mice is not associated with alterations in weight (12,13). GLP-1 exerts its central and peripheral effects via a specific GLP-1 receptor (GLP-1R). Genes encoding the nuclear factors that regulate proglucagon gene transcription, such as the LIM/homeodomain islet-1 gene (Isl-1) (14) may also be of interest, since proglucagon is the common precursor of glucagon and glucagon-like peptides (13).

Second, we studied gene products that modulate insulin action and glucose metabolism in target tissues. These gene products may contribute to the excess of fat deposition and the development of insulin resistance associated with obesity. They include the fatty acid–binding protein 2 (FABP-2) and

the insulin receptor substrate-1 (IRS-1). Previous studies have suggested that a mutation in FABP-2 may affect glucose uptake in Pima Indians (15,16). IRS-1 mediates most of insulin's effects and is also a candidate gene for insulin resistance and obesity (17).We have also tested the caudal-type homeodomain 3 gene (CDX-3), an insulin (18) and proglucagon gene transactivator (13).

Third, we looked at genes whose products are involved in energy expenditure and adipose tissue metabolism. Lipoprotein lipase (LPL) provides fatty acids for fat storage in adipose tissue and interacts with a co-activator apoprotein-C2 (apo-C2) (19). The hormone-sensitive lipase (HSL) is one of the enzymes that determines whole-body fuel availability by catalyzing the rate-limiting step of adipose tissue lipolysis (20). The peroxisome proliferator-activated receptor-γ (PPAR-γ) is an important transcription factor activated by fatty acids that transactivates several genes involved in adipocyte metabolism and is critical for adipocyte differentiation (21). An overexpression of tumor necrosis factor-α (TNF-α) was reported in adipose tissue of rodent models of obesity (22) and obese subjects (23). In Pima Indians, TNF-α locus was linked to body fat (24). The liver carnitine palmitoyltransferase-1 (CPT-1) is the key enzyme of long-chain fatty acid transportation into the mitochondria before oxidation (25). Polymorphism in the uncoupling protein-1 (UCP-1) and the β_3-adrenoceptor (β_3-AR) were associated with weight gain (26,27) and obesity-related phenotypes in several unrelated subjects, but these loci were not tested in familial linkage studies in very obese Caucasians.

RESEARCH DESIGN AND METHODS

Subjects and phenotypes. DNA and clinical data were collected by two different sources: through an individual-oriented multimedia campaign in the French population (group 1) and from the Department of Nutrition of the Hôtel-Dieu Hospital in Paris (group 2) (8). The familial inclusion criterion was the presence in the nuclear family of one morbidly obese subject with a BMI >40 kg/m^2 and at least one sib with a BMI 27 kg/m^2. Previous studies have suggested major gene effects in families ascertained through morbidly obese probands (28).

For linkage analyses, all affected and nonaffected available siblings and parents were included, resulting in 55 nuclear families with both parents, 69 nuclear families with one parent, and 54 without parents. Clinical and metabolic data were obtained for each obese and nonobese subject during an examination performed at Hôtel-Dieu Hospital or by the subject's personal physician (8).

Biochemical analysis. Leptin levels were measured after an overnight fasting (Kit Linco; Linco Research, St. Louis, MO). Plasma glycerol and free fatty acids were determined by a sensitive radiometric method (29) and an enzymatic procedure (Unipath, Dardilly, France).

DNA analyses. Subjects were genotyped using simple tandem repeat DNA polymorphisms located in the genes for Isl-1, FABP-2, GLP-1R, CDX-3, IRS-1, and CCK-BR (30,31) or nearby markers for β_3-AR (32), CCK-AR (33), TNF-α (24), and apo-C2. For the UCP gene, an A G polymorphism of the promotor (27) and an intragenic microsatellite were tested. The CPT-1 and PPAR-γ genes were mapped on chromosomes 11 q13 (25) and 3 (34), respectively. For HSL (35) (36) and LPL genes (37), both intragenic and nearby markers were tested (Table 1). In the region showing an indication for linkage, the other two flanking markers at the Isl-1 locus (D5S418 and D5S407) were typed as described (30,31).

Linkageanalyses. For nonparametric-affected sib-pair linkage analyses, we calculated π, the mean proportion of marker alleles shared identical-by-descent among affected sib pairs using a one-sided *t* test. π should be greater than 0.5 in case of linkage. π was determined in all affected sib pairs (BMI 27 kg/m^2) for each marker in both sets of families (groups 1 and 2), in the two groups combined, and in siblings with BMI 30 and 35 kg/m^2 to obtain potentially more homogeneous subgroups. For each subject we calculated the *z* score (variation of the BMI compared with a sex- and age-matched French reference population). Subjects with a *z* score >1 and 2 (1 and 2 SD from the mean BMI of the French population) were considered as affected. For each marker loci, we determined empirical distributions of the statistical test using simulations. We simulated the transmission of a marker with the same informativity among affected subjects, with phenotypes (status or value) kept fixed (5,000 replicates used) (38).

TABLE 1
Markers and loci tested in the genetic linkage study

Genes (symbol)	Markers	Chromosome
β_3-AR	ANK1	8q12
UCP	FGFR1	
	D8S532	
	A G variant	4q28–q31
PPAR-γ	D4S420	
	D3S1263	3
HSL	LHS-A	19q13.1–q13.2
LPL	LHS-B	
	D19S223	
	Intragenic	8p22
Apo-C2	D8S261	
	apo-C2	19q13
TNF-α	D6S273	6p21.3
CCK-AR	D6S291	
	D4S491	4p15.1–p15.2
CCK-BR	CCKB-R	11p15
GLP1-R	GLP1-R	6p21
FABP-2	FABP2	4q28–q31
CDX-3	CDX3	13q12
IRS1	IRS-1	2p35
Isl-1	Isl1	5q
CPT-1	D11S987	11q13.1

We searched for possible linkages between markers and quantitative traits related to obesity such as BMI, *z* score for BMI, velocity of subject's weight gain ([weight] – [weight at 20 years] ÷ [age – 20]), fasting leptin, insulin, glycerol, and free fatty acids. The phenotypical values were adjusted for age and sex and other covariates' effects through multiple regressions before the analysis. The accuracy of the *P* value was evaluated through permutation procedures (39). Our PERL program randomly resorted the Ys among the actual πs 10,000 times for each loci. The 10,000 β slopes given by the test provided an empirical distribution. The probability of obtaining a β lower than the β observed at each locus in the study was determined (*P*-all). We also performed the test for all independent pairs (i.e., the pairs formed by the first sib and the other sibs of the family), deriving a *P* value we called *P*-ind. Multipoint linkage analyses between two additional markers at the Isl-1 locus were also performed using MAPMAKER/SIBS, providing a logarithm of odds (LOD) score.

RESULTS

We found no evidence for linkage of most of the tested genes with regard to all affected obese sib pairs or the subsets stratified according to obesity status (π0.5 for BMI ≥27 and ≥30 kg/m^2; ≥35 kg/m^2 not shown) (Table 2). An excess of allele sharing was found between obesity and some loci: IRS-1 in group 1 (π = 0.54) and CPT-1 in group 2 (π = 0.57), but this result was not replicated in the other set of families and no trend of linkage was observed with the two groups combined. When the obesity status was based on the *z* score, similar observations were made except for the Isl-1 locus. A weak indication for linkage was obtained for all affected sib pairs with a *z* score >1 SD of BMI (n = 226 sib pairs, π = 0.54 ± 0.02, P = 0.03).

As shown in Table 3, no evidence for linkage of the traits quantitatively related to obesity was observed for most of the tested loci for groups 1 and 2 combined. In group 1, a tendency toward linkage was observed for BMI with the three markers at the HSL locus (HSL-A: t = –1.86, P = 0.03 for n = 152 sib pairs; HSL-B: t = –1.85, P = 0.03, n = 172; D19S223: t = –2.30, P = 0.01 in n = 156 sib pairs), but these results were not repli-

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TABLE 2
Results of the sib-pair linkage analysis for affected sib pairs with BMI ≥27 kg/m^2

Marker	Group 1 π/P	Group 2 π/P	All $n/\pi/P$
β_3-AR			
ANK1	0.52/0.2	0.50/0.4	201/ 0.51/0.2
FGFR1	0.53/0.2	0.49/1	199/ 0.51/0.3
D8S532	0.53/0.2	0.50/1	177/0.52/0.3
UCP			
A G variant	0.54/0.01*	0.45/1	148/0.50/1
D4S420	0.49/1	0.46/1	175/0.48/1
PPAR-γ			
D3S1263	0.47/1	0.52/0.2	150/0.50/1
HSL			
HSL-A	0.54/0.1	0.50/0.5	160/0.52/0.2
HSL-B	0.48/1	0.53/0.2	168/0.51/0.4
D19S223	0.50/0.4	0.55/0.05*	145/0.53/0.1
LPL			
Intragenic	0.45/0.6	0.51/0.3	186/0.48/0.6
D8S261	0.45/1	0.46/1	194/0.45/1
Apo-C2	0.50/1	0.51/0.4	159/0.50/0.4
TNF-α			
D6S273	0.49/1	0.48/1	183/0.49/1
D6S291	0.50/1	0.50/1	172/0.50/1
CCK-AR			
D4S491	0.48/1	0.48/0.1	175/0.48/1
CCK-BR	0.47/0.1	0.50/1	160/0.51/1
GLP-1R	0.51/0.3	0.51/0.4	170/0.51/0.3
FABP-2	0.47/1	0.49/1	192/0.48/1
CDX-3	0.49/1	0.47/1	143/0.48/1
IRS-1	0.54/0.02*	0.50/1	195/0.52/0.1
Isl-1	0.54/0.04*	0.50/1	186/0.52/0.1
CPT-1			
D11S 987	0.48/1	0.57/0.009	186/0.53/0.1

SDs are similar (between 0.02 and 0.04) for each π. n, number of affected sib pairs. *$P \geq 0.05$.

TABLE 3
Results of the sib-pair linkage analysis for BMI and leptin

Marker	Number of pairs	ln BMI t/P	Ln Leptin t/P
β_3-AR			
ANK1	315	0.19/NS	0.1/NS
FGFR1	300	–0.4/NS	–0.1/NS
D8S535	380	1.1/NS	1.0/NS
UCP			
A G variant	203	–2.26/0.01	–1.34/0.09
D4S420	283	0.5/NS	–1.24/01
PPAR-γ			
D3S1263	306	0.9/NS	1.83/NS
HSL			
HSL-A	241	–1.06/0.1	–0.95/0.1
HSL-B	279	–1.06/0.1	–0.64/0.2
D19S223	232	–0.37/NS	0.05/NS
LPL			
Intragenic	400	1.8/NS	0.6/NS
D8S261	320	1.27/NS	0.15/NS
Apo-C2	225	–1/0.15	–0.61/NS
TNF-α			
D6S273	306	0.003/NS	0.39/NS
D6S291	286	–1.33/0.09	–0.14/NS
CCK-AR			
D4S491	279	0.27/NS	0.6/NS
CCK-BR	221	–0.40/NS	–2.18/0.01
GLP-1R	400	0.51/NS	2.3/NS
FABP- 2	480	–0.50/NS	0.05/NS
CDX-3	206	–0.05/NS	0.29/NS
IRS-1	435	–1.06/0.1	–1.45/NS
Isl-1	284	–2.5/0.006	–3.4/0.0004
CPT-1			
D11S 987	401	1.23/NS	–0.12/NS

Analyses were performed after log transformation of BMI and leptin.

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cated in group 2 and were not found with the other quantitative traits. A similar indication for linkage was found for CCK-BR (t = –2.86, P = 0.0024 for BMI in 153 sib pairs and t = –2.186, P = 0.01 for leptin in 70 sib pairs), but this result was not replicated in group 2. We failed to observe any linkage between β_3 and UCP-1 loci and weight gain, plasma glycerol, and free fatty acids levels. Similar negative results were obtained when analyzing FABP-2 and IRS-1 loci with fasting insulinemia.

In contrast, a suggestive indication for linkage was found between the Isl-1 locus and BMI and leptin (P = 0.006 and 0.0004, respectively), adjusted for age and sex (Table 3). The empirical permutation P values were 0.0001 and 0.0000025, the P-ind value was 0.0000075, and the P-all values were 0.005 and 0.0001, respectively. This indication for linkage was particularly due to the results obtained in group 1 (t = –2.73, P = 0.002 for BMI and t = –2.55, P = 0.0005 for leptin); similar trends were observed in group 2 (t = –1.67, P = 0.048 for BMI and t = –2.10, P = 0.02 for leptin). After adjustments of leptin levels for age, sex, and BMI, the Isl-1 locus remained significantly linked to leptin in both groups (P = 0.003 in group 1 and P = 0.009 in group 2) and in the two groups combined (P = 0.0001). These trends for linkage with Isl-1 were also found with glycerol (t = –1.86, P = 0.03) and free fatty acid levels (t = –1.65, P = 0.04). We typed two additional markers close to the Isl-1 locus (D5S418 and D5S407 at 2.7 and 4 cM, respectively). Trends for linkage were found with leptin and these markers (D5S418: t = –3.0, P = 0.001; D5S407: t = –1.0, P = 0.1). Multipoint analyses using these flanking markers and Isl-1 showed that the LOD score was 1.73, coinciding with Isl-1 locus (Fig. 1).

DISCUSSION

Using linkage analyses in the French families, we attempted to find a gene with a genotypic risk ratio of four and a moderate deleterious allele frequency (40). Because of the selection procedure (i.e., via multiplex obese families with morbidly obese individuals in the sibship), a slightly greater power is expected. Most of the loci gave no significant evidence for linkage, suggesting that these genes are not major contributors for obesity in this population. The best evidence for linkage was observed between the Isl-1 locus on chromosome 5q and BMI and plasma leptin levels. The P value (P = 0.0001) observed with leptin is generally thought to be sufficient to suggest linkage with a candidate gene. Moreover, we used empirical distributions to control potential inflation of

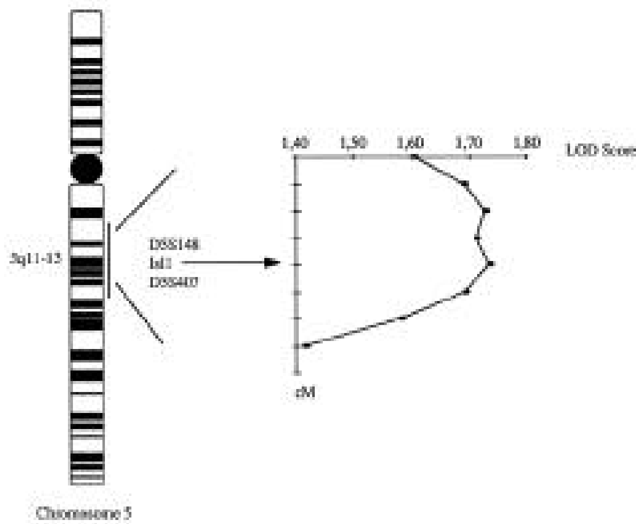

FIG. 1. LOD score for ln leptin at Isl-1 locus on chromosome 5q. The *y*-axis shows the LOD score calculated by the MAPMAKER/SIB program at 1-cM interval. The *x*-axis corresponds to the genetic distance of markers calculated by the MAPMAKER program as percent recombination (cM). The putative position of each marker is indicated by an arrow.

the *P* value. The marker-wise empirical *P* values either equaled the corresponding nominals or were even lower for very significant nominals. The low experiment-wise *P* values strengthened the conclusion that these results were unlikely to occur by chance for any marker. To address the question of dependence of the pairs in large sibships, we repeated the permutation robust test for the independent pairs and the *P* values remained in the same range. We also found a modest indication for linkage with obesity for affected sib pairs with a *z* score >1 SD of BMI. Although all our obese families met the same criteria for inclusion, they were recruited from two independent sources (hospital versus free voluntary participation), making possible the comparison of linkage data between the two groups. The indication for linkage at the Isl-1 locus in group 1 was associated with a similar trend in group 2, which also reinforces our results. Taken together, these data and the results of the multipoint analysis performed with Isl-1 and two other flanking markers (LOD score = 1.73) suggest the existence of a putative obesity susceptibility gene located at or near the Isl-1 locus.

Isl-1, as a member of a family of proteins with a homeobox domain, is required for the development of all endocrine islet cells (41). Isl-1 functions as a positive regulator of proglucagon gene transcription. Members of this peptide family are expressed in a highly specific manner in the brain, intestine, and pancreatic islets and are key regulators of carbohydrate, lipid, and protein metabolism (13). Moreover, GLPs have been implicated as mediators of the glucose-dependent insulin secretion, a process that might be disturbed in the evolution of obesity. The role of the GLP-1/GLP-1R pathway in feeding behavior remains a debated question (12,13). Screening of the Isl-1 gene for mutations in obese patients in linked families will allow us to evaluate its putative role in obesity. Alternatively, Isl-1 polymorphism might serve as a marker to an obesity gene lying in its vicinity.

We did not find any evidence of linkage between the TNF-α locus and adiposity in our population, in contrast to previous data in Pima Indians (24). This might be due to ethnic differences or to our indirect assessment of the fat mass through BMI and leptin measurements. We previously have found associations between the β_3-adrenoceptor and UCP polymorphisms and weight gain (26,27), but we failed to detect any linkage between this phenotype and β_3-AR and UCP loci in the families. Genetic variations located in IRS-1 (17), FABP-2 (15,16), and HSL-A (35) loci have been associated with features of insulin resistance in Caucasians and Pima Indians, but we did not see any linkage between those loci and fasting insulinemia, which is a marker of insulin resistance. Because sib-pair linkage analysis has moderate ability to detect genes with minor effects on complex traits, it cannot be excluded that variations in such genes may participate in the polygenic background of obesity. The role of susceptibility genes (neither sufficient nor necessary for multifactorial diseases) might be detected only by association studies (42) and/or by transmission disequilibrium, which could explain the discrepancy between our present data and previous reports on these candidate genes.

In summary, 14 out of the 15 tested candidate genes did not meet the criteria for a major obesity susceptibility gene. Whether one or several genes on chromosome 5q (located at the islet-1 locus) play a role in the pathogenesis of obesity in the families we studied merits further examination.

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