Am. J. Hum. Genet. 61:668–677, 1997

Recessive Inheritance of Obesity in Familial Non–Insulin-Dependent Diabetes Mellitus, and Lack of Linkage to Nine Candidate Genes

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Summary

Segregation analysis of body-mass index (BMI) supported recessive inheritance of obesity, in pedigrees ascertained through siblings with non–insulin dependent diabetes mellitus (NIDDM). BMI was estimated as 39 kg/m^2 for those subjects homozygous at the inferred locus. Two-locus segregation analysis provided weak support for a second recessive locus, with BMI estimated as 32 kg/m^2 for homozygotes. NIDDM prevalence was increased among those subjects presumed to be homozygous at either locus. Using both parametric and nonparametric methods, we found no evidence of linkage of obesity to any of nine candidate genes/regions, including the Prader-Willi chromosomal region (*PWS*), the human homologue of the mouse *agouti* gene (*ASP*), and the genes for leptin (*OB*), the leptin receptor (*OBR/DB*), the β_3-adrenergic receptor (*ADRB3*), lipoprotein lipase (*LPL*), hepatic lipase (*LIPC*), glycogen synthase (*GYS*), and tumor necrosis factor α (*TNFA*).

Introduction

Obesity is one of the strongest risk factors for non–insulin dependent diabetes mellitus (NIDDM) (Hansen 1995). In rodents, obesity and diabetes co-occur as pleiotropic effects of several genetic defects (Bouchard 1995). Likewise, in humans, obesity and NIDDM may co-occur as pleiotropic effects of a single gene. On the other hand, lean individuals also develop NIDDM, including lean relatives of obese NIDDM patients. Consequently, another possibility is that obesity, regardless of cause, increases the risk of NIDDM in susceptible individuals. Therefore, the inherited obesity expressed in pedigrees selected through cases of NIDDM may result from genes that predispose for both NIDDM and obesity, from genes that predispose only for obesity, or from genes of both types.

Prader-Willi syndrome is one known genetic cause of obesity; the syndrome also includes short stature, small hands and feet, a characteristic face, mental deficiency, hypotonia, and hypogonadism (Donaldson et al. 1994). Most patients have a deletion on the long arm of the paternally inherited chromosome 15. The deletion undoubtedly encompasses multiple genes, each of which is responsible for a subset of the component phenotypes of the syndrome. A mutation in a single gene within the deleted region may produce nonsyndromal obesity. However, such a gene has yet to be identified.

Although the genes that underlie human obesity have yet to be identified, the human homologues of mouse obesity genes are reasonable candidates. A dominant mutation in the *agouti* gene produces obesity and diabetes, as well as a yellow coat color, compared with the wild-type phenotype of banded black and yellow hairs (Yen et al. 1994). The *ob* and *db* genes code for leptin and the leptin receptor, respectively; a recessive mutation in either produces obesity and strain-specific diabetes, in rodents (Zhang et al. 1994; Lee et al. 1996).

Other obesity candidate genes play a role in lipolysis, glycogen synthesis, and insulin resistance. The β_3-adrenergic receptor is thought to affect fatty-acid mobilization (Emorine et al. 1994). Lipoprotein lipase (Eckel 1989) and hepatic triglyceride lipase are thought to provide fatty acid for storage in adipose tissue. Glycogen synthase is the rate-limiting insulin-sensing enzyme in glucose storage (Felber et al. 1993). Tumor necrosis factor α plays a role in the insulin resistance of obesity and of NIDDM (Hotamisligil and Spiegelman 1994).

In this study, we used segregation analysis to test for major-locus inheritance of obesity in 42 pedigrees ascertained through siblings with NIDDM. Then, we tested for linkage between obesity and nine obesity candidate genes/regions, including the Prader-Willi chromosomal region (*PWS*), the human homologue of the mouse *agouti* gene (*ASP*), and the genes for leptin (*OB*), the leptin receptor (*OBR/DB*), the β_3-adrenergic receptor (*ADRB3*), lipoprotein lipase (*LPL*), hepatic lipase (*LIPC*), glycogen synthase (*GYS*), and tumor necrosis factor α (*TNFA*).

Received November 5, 1996; accepted for publication June 6, 1997.

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0002-9297/97/6103-0026$02.00

Subjects and Methods

We ascertained 42 pedigrees that met our criteria of at least two siblings with onset of NIDDM before age 65 years and at most one parent known to have NIDDM. First-, second-, and third-degree relatives of the probands were studied when available. All sample members were of northern European ancestry. We measured height and weight and performed a standard 2-h, 75-g oral glucose-tolerance test with fasting and 1-h insulin levels. We computed the body-mass index (BMI) as weight divided by height squared. Glucose level was measured by a standard glucose oxidase assay; one of two laboratories measured insulin levels by double-antibody radioimmunoassay. BMI and fasting, 1-h, and 2-h glucose levels were age- and gender-adjusted by use of regression; fasting and 1-h insulin levels were adjusted for age, gender, and the testing laboratory, by use of regression. BMI measurements were available for a total of 616 individuals, within the range of 1–37 individuals per pedigree. Each participant in the study gave informed consent. This study was approved by the Institutional Review Board of the University of Utah Health Sciences Center, Salt Lake City.

Microsatellite markers were amplified from 60 ng of DNA, by use of $\gamma[^{32}P]$-labeled primer. Autoradiographs were read by two individuals. Consistency of scoring was maintained by the running of control samples across all gels. Allele frequencies were estimated by use of a sample of ~100 unrelated individuals, most of whom are spouses of pedigree members.

We used likelihood analysis to test for major-locus inheritance of high levels of BMI. We computed the likelihoods of the genetic models (Elston and Stewart 1971), using PAP (Hasstedt 1994), and obtained the maxima, using NPSOL (Gill et al. 1986). We corrected for the ascertainment of each pedigree, through a sib pair with NIDDM and through a parent not known to have NIDDM, by dividing each pedigree likelihood by the likelihood of the measured BMI for these individuals (Young et al. 1988). We tested significance using χ^2 statistics. Under certain conditions, the natural logarithm of the ratio of the likelihood of a submodel relative to the likelihood of a general model, multiplied by -2, approximated a χ^2 distribution. The χ^2 test had df equal to the number of parameters restricted when the submodel was specified from the general model.

The genetic model used in the analysis specified each phenotype as the sum of independent effects attributed to the segregation of alleles at major loci, the transmission of polygenes, and random factors specific to the individual. This analysis extended to multiple loci the standard mixed model that includes a single major locus and polygenes (Elston and Stewart 1971; Morton and MacLean 1974). We assumed each major locus had two alleles in Hardy-Weinberg equilibrium. The polygenic and random environmental components were assumed to be normally distributed within genotypes. When the model included polygenes and one or more major loci, the likelihood was approximated (Hasstedt 1993).

The parameters of the model included the total mean (μ), the total standard deviation (σ), the frequency of the allele determining high BMI at locus L (q_L), the dominance at locus L (d_L), the displacement at locus L (t_L), polygenic heritability (h^2), and parent-to-offspring transmission probabilities (τ_1, τ_2, and τ_3) for the three genotypes at one locus (Boyle and Elston 1979; Lalouel et al. 1983). Displacement is the difference, in within-genotype SDs, between the means for the two types of homozygotes. Dominance is the difference between the mean for heterozygotes and the mean for homozygotes, for low BMI relative to the displacement. We assumed additivity of displacement across loci; that is, for a two-locus model, the displacement for both loci together equaled the sum of the displacement at locus 1 and at locus 2. The polygenic heritability is the proportion of the variance within major-locus genotypes, owing to polygenic inheritance. Mendelian inheritance specifies $\tau_1 = 1$, $\tau_2 = .5$, and $\tau_3 = 0$.

We inferred major loci sequentially. We first tested for one major locus by specifying the most general model, through the parameters μ, σ, q_1, d_1, t_1, h^2, τ_1, τ_2, and τ_3. When one locus was inferred, we tested for a second locus by specifying the most general model, through the parameters μ, σ, q_1, t_1, q_2, d_2, t_2, h^2, τ_1, τ_2, and τ_3; by fixing d_1 to its estimate in the one-locus model; by assuming Mendelian transmission at locus 1; and by applying τ_1, τ_2, and τ_3 to locus 2. When two loci were inferred, we tested for a third locus by specifying the most general model, through the parameters μ, σ, q_1, t_1, q_2, t_2, q_3, d_3, t_3, h^2, τ_1, τ_2, and τ_3; by fixing d_1 and d_2 to their estimates in the two-locus model; by assuming Mendelian transmission at locus 1 and at locus 2; and by applying τ_1, τ_2, and τ_3 to locus 3. The test of no major locus L compared the likelihood of $q_L = 0$ to the likelihood of q_L estimated, with the restriction that $\tau_1 = 1$, $\tau_2 = .5$, and $\tau_3 = 0$. For the first locus, this test compared the likelihood of a one-locus model to the likelihood of a polygenic model; for the second locus, this test compared the likelihood of a two-locus model to the likelihood of a one-locus model; and for the third locus, this test compared the likelihood of a three-locus model to the likelihood of a two-locus model. The tests of Mendelian transmission and of environmental nontransmission, at locus L, compared the likelihood of $\tau_1 = 1$, $\tau_2 = .5$, and $\tau_3 = 0$ and the likelihood of $1 - q_L = \tau_1 = \tau_2 = \tau_3$, respectively, with the likelihood of estimated τ_1, τ_2, and τ_3. We inferred major-locus inheritance when we rejected the hypotheses of no major locus and of environmental nontransmission but did not

reject Mendelian transmission. The tests of recessivity and of dominance, at locus L, compared the likelihood of $d_L = 0$ and the likelihood of $d_L = 1$, respectively, with the likelihood of d_L estimated, with the restriction that $\tau_1 = 1$, $\tau_2 = .5$, and $\tau_3 = 0$.

We used genotypic probability estimators (GPEs) (Hasstedt and Moll 1989) to estimate the effects of the BMI major loci on other variables. To use GPEs, one partially assigns a genotype to each individual, using a genotypic probability p_{ij}—that is, the probability that person i has genotype j—which equals the relative likelihood of the genetic model conditioning on person i having genotype j. The parameters of the model were fixed at their maximum-likelihood estimates for the inferred genetic model. We then estimated the number of individuals with each genotype, within subgroups of the sample, as $n_j = \Sigma_i p_{ij}$ and the genotypic mean of variable x as $\hat{\mu}_j = \Sigma_i p_{ij}(x_i/n_j)$, where x_i equals the value of variable x measured for person i and where the summation is over all members of the subgroup. The estimates should be interpreted with caution; the assumption of independence is violated if residual genetic variation is present.

We tested for linkage to each of the obesity candidate genes, using the pseudomultipoint procedure in FASTMAP (Curtis and Gurling 1993) on single-marker LOD scores computed by PAP (Hasstedt 1994). The PAP LOD scores assumed the parameter estimates for the inferred two-locus genetic model for BMI and were computed for recombination fractions of 0% and 10%, for each obesity locus and each marker. In addition, we used MAPMAKER/SIBS (Kruglyak and Lander 1995) to compute multipoint LOD scores, using maximum-likelihood variance estimation and multipoint Z scores, using a nonparametric quantitative-trait-loci (QTL) method; this analysis required splitting the pedigrees into nuclear families.

We used likelihood analysis and the admixture model (Smith 1961; Ott 1983) to estimate the proportion of linked pedigrees and to compute the heterogeneity LOD score. The heterogeneity LOD score equaled $\text{LOD}_\alpha = \Sigma_i \log_{10}[\alpha L_i(c) + (1 - \alpha)]$, where α represents the proportion of linked pedigrees and $L_i(c)$ represents the antilog of the pseudomultipoint LOD score for pedigree i, at location c, and where the summation is over all pedigrees.

Results

Evidence supporting major-locus inheritance of obesity was derived first from the rejection of the hypothesis of no major locus ($\chi^2_{(3)} = 101.37$; $P < .0001$) and then from the rejection of environmental nontransmission ($\chi^2_{(3)} = 16.17$; $P = .001$), while Mendelian transmission failed to be rejected ($\chi^2_{(3)} = 5.74$; $P > .05$). This last χ^2 statistic indicates that the estimated transmission probabilities of $\tau_1 = .908 \pm .043$, $\tau_2 = .410 \pm .073$, and $\tau_3 = .000$ did not differ significantly from the Mendelian probabilities of 1, .5, and 0, respectively. We inferred recessive inheritance by rejecting dominance ($\chi^2_{(1)} = 46.47$; $P < .0001$) and by not rejecting recessivity ($\chi^2_{(1)} = 2.01$; $P > .05$). We designated this locus, with displacement estimated as 3.23 SDs, the "extreme-obesity locus."

Allowing for the extreme-obesity locus, we obtained evidence consistent with the existence of an additional locus with a more moderate effect, by rejecting the hypothesis of no second locus ($\chi^2_{(3)} = 32.74$; $P < .0001$), while also not rejecting Mendelian transmission ($\chi^2_{(3)} = 1.26$; $P > .05$), although we failed to reject environmental nontransmission ($\chi^2_{(3)} = 5.00$; $P = .17$). The transmission probabilities were estimated as $\tau_1 = .893 \pm .093$, $\tau_2 = .541 \pm .080$, and $\tau_3 = .034 \pm .040$. We inferred recessive inheritance by rejecting dominance ($\chi^2_{(1)} = 11.43$; $P < .001$) and by not rejecting recessivity ($\chi^2_{(1)} = 0.00$; $P > .05$). We designated this locus, with displacement estimated as 2.20 SDs, the "moderate-obesity locus."

We retested for the extreme-obesity locus, while allowing for a moderate-obesity locus. Again, we rejected environmental nontransmission ($\chi^2_{(3)} = 19.46$; $P < .001$), while failing to reject Mendelian transmission ($\chi^2_{(3)} = 6.29$; $P > .05$). The transmission probabilities were estimated as $\tau_1 = .911 \pm .059$, $\tau_2 = .411 \pm .132$, and $\tau_3 = .000$.

No evidence for a third locus determining BMI was obtained ($\chi^2_{(3)} = 0.64$; $P > .05$). The two-locus recessive model accounted for 68% of the variance in BMI. The remaining 32% of the variance was attributed to random environmental effects specific to each individual; we did not include in the model an environmental effect shared by siblings, since no siblings in this adult sample currently cohabit. The maximum-likelihood estimates, with standard errors, for the two-locus recessive model for standardized BMI were $q_M = .423 \pm .030$, $q_E = .284 \pm .008$, $t_M = 2.20 \pm 0.23$, $t_E = 4.47 \pm 0.18$, and $h^2 = .000$, where subscripts "M" and "E" designate the moderate-obesity and the extreme-obesity loci, respectively. A heritability estimate of 33% for the one-locus model agreed with other analyses of BMI; the addition of a second locus accounted for that genetic variation and reduced the estimate to .000.

Table 1 shows that the homozygosity of the inferred genes, for moderate obesity and for extreme obesity, resulted in a mean BMI of 32 kg/m^2 and of 39 kg/m^2, respectively. Our assumption of additive displacement across loci required that, over the normal genotype, the increase in BMI owing to the homozygosity of both genes equal the sum of the increases owing to the homozygosity of each gene alone. Although this assumption

Table 1

Number, Means, and Percentages, by Gender and Genotype Class, for the Complete Sample

	Men[a]			Women[a]			Total[a]		
Variable	N	M	E	N	M	E	N	M	E
No.	206	53	16	240	68	24	446	121	40
Mean age (years)	46	49	46	48	48	44	47	49	45
Mean BMI (kg/m^2)	25	31***	39***	24	33***	39***	25	32***	39***
With NIDDM (%)	21	41*	44	25	46**	45	23	44***	44
Mean age at onset of NIDDM (years)	53	50	47	52	49	44	53	50	45
With NIDDM, age $\geq$ 50 years (%)	43	71*	91	49	77*	94	46	75**	93

[a] Data were estimated by use of GPEs; the BMI was adjusted to a male of age 30 years. N = two-locus genotypes that are not homozygous for either obesity locus; M = two-locus genotypes that are homozygous at the moderate-obesity locus but not at the extreme-obesity locus; and E = two-locus genotypes that are homozygous at the extreme-obesity locus but not at the moderate-obesity locus. The two-locus genotype that is homozygous at both loci is not included, because of small numbers.

* $P < .01$; ** $P < .001$; *** $P < .0001$. All P values were determined by a one-tail t-test compared with the previous genotype group, without correction for multiple testing.

was not tested, the parameter estimates from the two-locus model predicted that nine individuals with a mean BMI of 47 kg/m^2 would be homozygous for both genes, which is in close agreement with the nine individuals with a mean BMI of 48 kg/m^2, estimated from the data (results not shown). Figure 1 compares the four inferred distributions to the sample distribution.

A total of 162 members of these pedigrees were diagnosed with NIDDM; another 416 pedigree members showed no symptoms of NIDDM; and the remaining 38 pedigree members were either not studied or the diagnosis was equivocal. Table 1 shows that both inferred obesity genes double the prevalence of NIDDM. Although not significant, age at onset was earlier in those members homozygous for the moderate-obesity gene and earlier still in those members homozygous for the extreme-obesity gene. When the sample was restricted to the 244 pedigree members of age $\geq$50 years, a higher

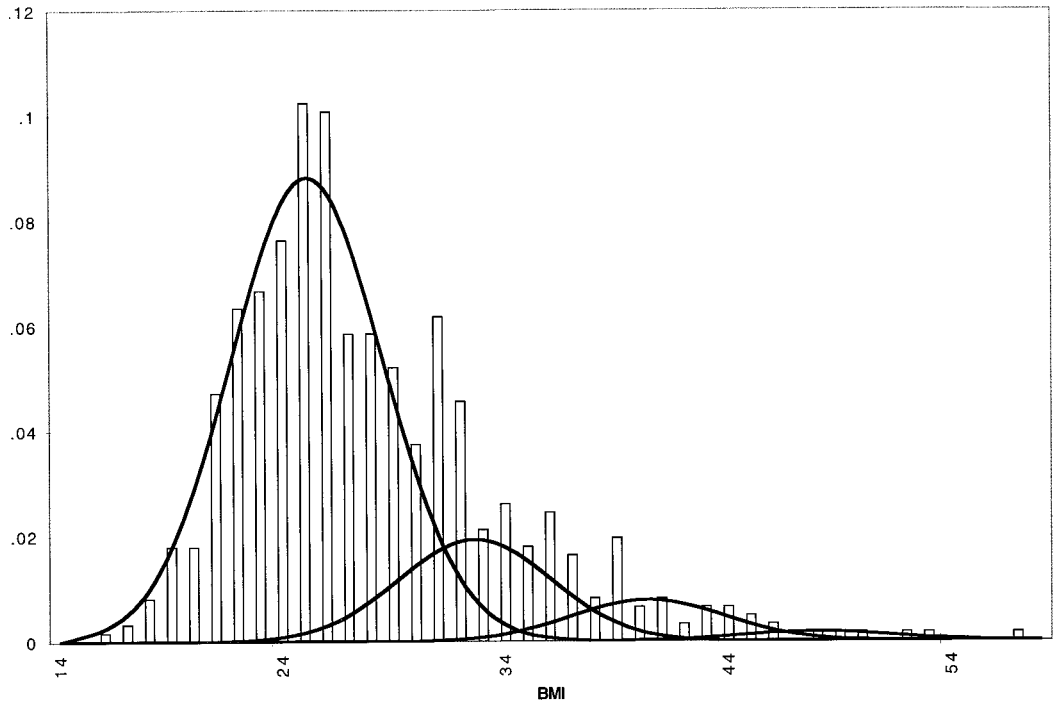

Figure 1 Distribution of BMI in the sample (bars) and for the inferred genetic model (four smooth curves)

Table 2

Mean Insulin and Glucose Levels, by Gender and Genotype Class, for Pedigree Members without NIDDM

Variable	No. of Members Tested	Mean, for[a]								
		Men			Women			Total		
		N	M	E	N	M	E	N	M	E
Insulin level (μU/ml):										
Fasting	383	13	21***	36**	13	19****	23	13	20****	28**
1-h	366	68	116***	213*	67	104***	129	68	110****	159**
Glucose level (mg/ml):										
Fasting	404	88	88	89	88	89	92	88	88	91
1-h	390	125	144**	147	126	139*	147	125	142**	147
2-h	391	88	100*	99	88	96*	99	88	98**	99

[a] Means were estimated by use of GPEs on natural logarithm–transformed measurements, adjusted to a male of age 30 years, then transformed to the original scale by use of the lognormal mean. N = two-locus genotypes that are not homozygous for either obesity locus; M = two-locus genotypes that are homozygous at the moderate-obesity locus but not at the extreme-obesity locus; and E = two-locus genotypes that are homozygous at the extreme-obesity locus but not at the moderate-obesity locus. The genotype that is homozygous at both loci is not included, because of small numbers.

* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$. All P values were determined by a one-tail t-test compared with the previous genotype group, by use of natural logarithm–transformed measurements, without correction for multiple testing.

prevalence of NIDDM was estimated for those members homozygous for the extreme-obesity gene than for those members homozygous for the moderate-obesity gene.

Table 2 shows that both inferred obesity genes increase fasting and 1-h insulin levels and 1-h and 2-h glucose levels but not fasting glucose levels. The effect of the genes on insulin levels is larger than the effect on glucose levels.

Table 3 lists the obesity candidate genes, and table 4 lists the corresponding genetic markers used in the linkage analysis. The markers had 6–15 alleles, with an average of 10 alleles, and heterozygosity was within the range of 55%–88%, with an average of 76%. LOD scores ranging from −16.16 to −4.29 (table 5) excluded all nine candidate genes as the sole extreme-obesity gene, in these pedigrees; LOD scores ranging from −4.15 to −1.91 (table 5) excluded seven candidate genes as the sole moderate-obesity gene and almost excluded the other two candidate genes. We cannot rule out one of the candidate genes being one of multiple extreme- or moderate-obesity genes, but the strongest evidence, a heterogeneity LOD score of 1.09 (table 5) for *ASP* and for the moderate-obesity locus, provided little support. Likewise the sib-pair analysis provided little support for a role for any of the candidate genes in the determination of BMI (table 5).

Discussion

The inference, in this study, of recessive inheritance of obesity agrees with other segregation analyses (Price et al. 1990; Moll et al. 1991; Ness et al. 1991; Borecki et al. 1993; Comuzzie et al. 1995). Nevertheless, one or both genes inferred herein may be different from those

Table 3

Designations and Locations of the Obesity Candidate Genes/Regions Tested for Linkage

Candidate Gene/Region	Symbol	Location	Reference
Leptin receptor	*OBR/DB*	1p31-1pter	Tartaglia et al. 1995
Tumor necrosis factor α	*TNFA*	6p21.3	Nedwin et al. 1985
Leptin	*OB*	7q31.3	Zhang et al. 1994
Lipoprotein lipase	*LPL*	8p22	Sparkes et al. 1987
β_3-adrenergic receptor	*ADRB3*	8p11-12	Bruskiewich et al. 1996
Prader-Willi	*PWS*	15q11-13	Magenis et al. 1990
Hepatic lipase	*LIPC*	15q21	Sparkes et al. 1987
Glycogen synthase	*GYS*	19q13.3	Lehto et al. 1993
Mouse *agouti* homologue	*ASP*	20q11.2	Kwon et al. 1994

Table 4

Genetic Markers Used to Test Linkage to the Obesity Candidate Genes/Regions

Candidate Gene/ Region	Genetic Marker(s)
OBR/DB	*D1S193, D1S168, D1S161, D1S162, D1S200*
TNFA	*D6S299, TNFA, D6S291*
OB	*D7S466, D7S514, D7S530*
LPL	*LPL*
ADRB3	*D8S87, FGFR1, D8S532*
PWS	*D15S128, D15S97, D15S165*
LIPC	*LIPC*
GYS	*GYS*
ASP	*D20S45, D20S106, SRC*

inferred in previous studies. First, a mean BMI of 39 kg/m^2 for the extreme-obesity locus exceeds previous estimates of 32–35 kg/m^2 (Price et al. 1990; Moll et al. 1991), although the estimate of 32 kg/m^2 for the less well-supported moderate-obesity locus does not. Second, some studies failed to infer recessive inheritance without the inclusion of genotype-specific gender and/or age effects (Tiret et al. 1992; Borecki et al. 1993; Comuzzie et al. 1995), which we did not find necessary. Third, we inferred genes for obesity in adulthood, but other samples included children (Price et al. 1990; Moll et al. 1991; Ness et al. 1991; Borecki et al. 1993): childhood obesity does not necessarily predict obesity in adulthood (Gasser et al. 1995). Finally, the loci inferred herein may produce obesity and NIDDM pleiotropically, which is less likely in the other samples, which were not ascertained through NIDDM cases.

Unfortunately, this analysis cannot distinguish between the two possibilities—that is, the inferred genes produce obesity and NIDDM pleiotropically, or the inferred genes produce obesity only, which increases the risk of NIDDM in susceptible individuals. The increased prevalence of NIDDM among individuals homozygous for the putative genes is consistent with either possibility. The hyperinsulinemia observed in nondiabetic homozygotes may predict the development of NIDDM consistent with pleiotropy but, instead, is probably simply a physiological correlate of obesity (Ferrannini 1995); fasting and 1-h insulin levels, after adjustment for BMI, showed no elevation in homozygotes (data not shown). Therefore, the recessive inheritance inferred for high fasting insulin levels, adjusted for BMI, in nondiabetic members of a subset of these pedigrees (Schumacher et al. 1992) undoubtedly is not due to either obesity gene inferred herein.

The question of whether the putative obesity genes produce both NIDDM and obesity pleiotropically or whether they produce obesity alone can be better answered when the genes have been identified. Despite the availability of parameter estimates to test for linkage to the candidate genes, using a genetic model, we also used a nonparametric method that does not require the specification of a genetic model, and we allowed for locus heterogeneity when using the parametric method; our conclusion, from the results of the segregation analysis, that obesity results from two major loci did not modify our expectation that multiple loci with different modes of inheritance underlie obesity; the consistent inference, by use of segregation analysis, of recessive inheritance may result partially from recent increases in the prevalence of obesity (Price et al. 1994). Unfortunately, none of the methods of linkage analysis implicated any of the tested obesity candidate genes, although we cannot rule

Table 5

LOD and *Z* Scores for Obesity Candidate Genes/Regions

Candidate Gene/ Region	Moderate-Obesity Gene[a]			Extreme-Obesity Gene[a]			Sib Pair[b]	
	LOD Score	α	LOD_α	LOD Score	α	LOD_α	LOD Score	*Z* Score
OBR/DB	−1.92	.25	.30	−13.20	.00	.00	.00	−.48
TNFA	−2.24	.25	.43	−9.63	.00	.00	.04	1.35
OB	−3.43	.00	.00	−14.21	.00	.00	.01	.06
LPL	−4.15	.00	.00	−6.65	.00	.00	.00	−.27
ADRB3	−3.73	.12	.05	−16.16	.00	.00	.05	1.23
PWS	−3.89	.08	.08	−7.91	.35	.50	.00	−.27
LIPC	−3.47	.00	.00	−4.29	.22	.22	.00	−.30
GYS	−3.98	.00	.00	−8.43	.00	.00	.04	.87
ASP	−1.91	.35	1.09	−12.85	.00	.00	.12	.78

Note.—The LOD and *Z* scores given are for tight linkage to the candidate gene when among the markers or, otherwise, for the highest LOD score or *Z* score between the two outside markers.

[a] α is the estimate of the proportion of linked pedigrees and LOD_α is the LOD score when heterogeneity is assumed.

[b] The maximum-likelihood variance LOD score and the nonparametric QTL *Z* score are given (Kruglyak et al. 1995).

out a rare defect, in one of the genes, underlying the obesity in only one or two of the pedigrees.

Other investigators have attempted to attribute variation in obesity, in NIDDM, or in related traits to genetic variation in the obesity candidate genes studied here, through linkage or association studies or through mutation screening. Of the obesity candidate genes considered here, *OB* is the most likely to be involved in human obesity, with a LOD score of 3.1 for linkage to extremity skinfold in Mexican-Americans (Duggirala et al. 1996), with suggestive evidence of linkage to obesity in the French (Clement et al. 1996), and with weak evidence of linkage to obesity in a United States sample (Reed et al. 1996), although linkage in Pima Indians was rejected (Norman et al. 1996). Nevertheless, a causative *OB* mutation has yet to be found in any obese subject (Considine et al. 1996*b;* Maffei et al. 1996; Niki et al. 1996), suggesting that if *OB* mutations exist in humans, then they are rare. In addition, NIDDM does not show linkage to *OB* (Stirling et al. 1995), even in the sample showing linkage to extremity skinfold (Duggirala et al. 1996).

After *OB, ADRB3* shows the strongest evidence of involvement in human obesity. The Trp64Arg mutation associates with obesity (Kadowaki et al. 1995; Kurabayashi et al. 1996), an increased capacity to gain weight (Clément et al. 1995; Fujisawa et al. 1996), low resting metabolic rates (Walston et al. 1995), abdominal obesity (Widén et al. 1995), and susceptibility to NIDDM (Fujisawa et al. 1996). Despite these associations, Candelore et al. (1996) found that *ADRB3* with the Trp64Arg mutation is pharmacologically and functionally indistinguishable from wild-type *ADRB3*. The effects of the Trp64Arg mutation are possibly more subtle than the tested effects, or the Trp64Arg mutation is not causative but is in linkage disequilibrium with a causative mutation. On the other hand, Li et al. (1996) found no evidence of association with obesity for the Trp64Arg mutation, in Sweden, and we found no evidence of association with obesity for the Trp64Arg mutation and no evidence of linkage of *ADRB3* to obesity or to NIDDM, in the pedigrees in this study (Elbein et al. 1996).

GYS also has been subjected to association studies but with much weaker support. Association of the A_2 allele of an Xba1 polymorphism, with individuals with NIDDM or with a family history of NIDDM, has been reported in a Finnish population (Groop et al. 1993; Schalin-Jäntti et al. 1996) but not in the French (Zouali et al. 1993) or the Japanese (Kadowaki et al. 1993). Association of a simple tandem repeat with NIDDM has been reported in Japan (Kuroyama et al. 1994) but has not been found by others (Shimokawa et al. 1994; Hamada et al. 1995), and association of other polymorphisms have not been found (Bjørbæk et al. 1994). Linkage to NIDDM is not supported (Elbein et al. 1994*a*).

Homozygous lipoprotein lipase deficiency causes massive accumulation of chylomicrons in plasma and a corresponding increase of plasma triglyceride concentration; patients present in childhood with abdominal pain and pancreatitis (Brunzell 1995). Heterozygotes may have moderate lipid abnormalities (Wilson et al. 1990; Miesenböck et al. 1993; Tenkanen et al. 1994). Likewise, other polymorphisms in *LPL* associate more strongly with lipid levels than with obesity or with NIDDM (Ahn et al. 1993; Elbein et al. 1994*b;* Jemaa et al. 1995; Ukkola et al. 1995), and linkage to fat mass (Comuzzie et al. 1995) and NIDDM (Elbein et al. 1995; Stern et al. 1996) has been rejected, although association with NIDDM has been reported (Wang et al. 1996). Homozygous hepatic lipase deficiency is much rarer than homozygous lipoprotein lipase deficiency. Heterozygotes have variable phenotypes, without a specific lipid abnormality (Hegele et al. 1993).

Among the other obesity candidate genes, linkage of *TNFA* to the percentage of body fat in Pima Indians is weakly supported (Norman et al. 1995), but no variability in the *TNFA* promoter has been found in NIDDM patients (Hamann et al. 1995), and we did not find linkage to NIDDM (Elbein et al. 1995). No evidence of linkage to obesity, to NIDDM, or to related traits has been found for *OBR/DB* (Considine et al. 1996*a;* Duggirala et al. 1996; Norman et al. 1996), *PWS* (Reed et al. 1995), or *ASP* (Xu et al. 1995; Duggirala et al. 1996; Norman et al. 1996).

Whether any of these nine candidate genes play a role in human obesity or in NIDDM is still an open question. Defects in *OB* may contribute to obesity in some populations, but that conclusion awaits confirmation. *ADRB3* may contribute to variation in obesity, but the effect appears to be small. This study rules out a major role in obesity in northern Europeans for either *OB* or *ADRB3* or for any of the other candidate genes that we tested. We also found no evidence, in these pedigrees, of linkage of NIDDM to any of the candidate genes.

Acknowledgments

This work was supported by the research service of the Department of Veterans Affairs and by grants DK39311 (to S.C.E.) and HD17463 (to S.J.H.) from the NIH (the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institute of Child Health and Human Development, respectively). Family ascertainment was supported by PHS grant M01-RR00064 from the National Center for Research Resources to the General Clinical Research Center, and, in part, by a Harold Rifkin Family Acquisition Center grant from the American Diabetes Association, as part of the Genetics of NIDDM project. Primer synthesis was supported, in part, from Cancer Center Support Grant P30 CA42014 and by Public Health Service grant 5-P30-HG00199 (to M.F.L.).

We thank Kimberley Wegner and Cindy Miles for patient sampling and Teresa Maxwell for preparation of DNA samples.

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