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Genome Scan for Human Obesity and Linkage to Markers in 20q13

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Summary

Obesity is a highly prevalent, multigenic trait that predicts increased morbidity and mortality. Here we report results from a genome scan based on 354 markers in 513 members of 92 nuclear families ascertained through extreme obesity and normal body weight. The average marker interval was ~10 cM. We examined four correlated obesity phenotypes, including the body-mass index (BMI) (both as a quantitative trait and as a discrete trait with a threshold of BMI $\geq$30 kg/m^2) and percentage of fat (both as a quantitative trait and as a discrete trait with a threshold of 40%) as assessed by bioelectrical impedance. In the initial stage of the genome scan, four markers in 20q gave positive evidence for linkage, which was consistent across most obesity phenotypes and analytic methods. After saturating 20q with additional markers (25 markers total) in an augmented sample of 713 members from 124 families, we found linkage to several markers in a region, 20q13, previously implicated in both human and animal studies. Three markers (D20S107, D20S211, and D20S149) in 20q13 had empirical *P* values (based on Monte Carlo simulations, which controlled for multiple testing) $\leq$.01 for single-point analysis. In addition, the parametric, affecteds-only analysis for D20S476 yielded a LOD score of 3.06 (*P* = .00009), and the affected-sib-pair test yielded a LOD score of 3.17 (*P* = .000067). Multipoint analyses further strengthened and localized these findings. This region includes several plausible candidate genes for obesity. Our results suggest that one or more genes affecting obesity are located in 20q13.

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Introduction

Obesity is an increasingly common trait that now affects nearly one-half of the U.S. adult population (Flegal et al. 1998). Obesity has serious consequences for health, including increased risks for non–insulin-dependent diabetes mellitus (NIDDM), hypertension, and cardiovascular diseases (Bray 1986). Family studies have demonstrated the heritable nature of obesity, and twin and adoption studies have shown that the familial pattern is due primarily to genetic factors (Price et al. 1987; Sørensen et al. 1989; Stunkard et al. 1990; Price and Gottesman 1991). The mode of inheritance is complex, and segregation studies have supported both polygenic inheritance and recessive-major-gene influence (Price et al. 1990; Moll et al. 1991; Borecki et al. 1998). There are several monogenic and polygenic animal models of obesity, as well as a number of candidate genes and regions drawn from human and animal studies (Chagnon et al. 1998).

Several major genes affecting obesity have been identified in mice (Tecott et al. 1995; Pomp et al. 1996; Dong et al. 1997; Good et al. 1997; Huszar et al. 1997; Chagnon et al. 1998). In humans, however, with rare exceptions (Jackson et al. 1997; Montague et al. 1997; Clement et al. 1998; Strobel et al. 1998), no clear relations between specific genotypes and obesity phenotypes have been found. Thus far, two groups have reported linkages in the context of a genome scan; one group reported suggestive linkage of fat percentage to markers in 11q21-q22 in Pima Indians (Norman et al. 1998), and the other group found positive linkage, for leptin levels and fat mass, with markers in 2p21 in Mexican Americans (Comuzzie et al. 1997).

Here we report results from an autosomal genome scan for measures of human obesity. After obtaining results of an initial scan, we selected one region (20q) that provided the most consistent support for linkage across phenotypes and across analytic methods. We saturated this region and found linkage of markers in 20q13 to quantitative and discrete indices of obesity. This region

includes markers linked to obesity in other samples, as well as several plausible candidate genes for obesity.

Families and Methods

Families

We studied nuclear families ascertained from an ongoing linkage study at the University of Pennsylvania. The sampling design and detailed sample characteristics have been described elsewhere (Price et al. 1998). In brief, families were ascertained through extremely obese probands (body-mass index [BMI] $\geq$40) and included additional obese siblings (BMI $\geq$30) and normal-weight siblings and parents (BMI <27). The subjects in these families had a wide range of obese and thin phenotypes (BMI 16.8–76.2 kg/m^2; percentage of fat [%fat] 10.2–65.2), with relatively early ages at onset (54.5% of affected individuals in whom age at onset of obesity was $\leq$20 years). Some families recruited during the early part of the study did not meet all the requirements. We divided one three-generation family into two nuclear families. During the first-stage genome scan, we included the first 92 nuclear families (91 kindreds). For saturation mapping of chromosome 20q, a region that was promising on the basis of the first-stage scan, we added 32 families collected after the genome scan was initiated (124 total nuclear families).

Obesity Phenotypes

We studied the BMI (kg/m^2) and %fat. We used BMI as an index of obesity, because it correlates with other obesity measures and was available on the largest number of individuals. For relatives at remote geographic locations who provided blood, we used self-measured or reported height and weight. These reports have been shown to be reasonably accurate (Reed and Price 1998*b*). We also examined %fat as a phenotype; %fat was measured by bioelectric impedance (BIA; Valhalla Scientific), which is an indirect measure of body composition. %Fat measures obtained from BIA correlate well with those obtained by the hydrodensitometry method (Price et al. 1998). We analyzed four correlated obesity phenotypes: both BMI and %fat as both quantitative and discrete traits.

Quantitative traits.—We used untransformed values of BMI and %fat for both stages of the genome scan. For saturation of chromosome 20q only, we used two additional phenotypes to examine potential confounding effects of sex and age on the linkage results. First, we used standardized residuals of BMI and %fat after controlling for sex and age by means of multiple linear regression. Second, we conducted a females-only analysis by coding the males' obesity phenotypes as unknown.

Discrete traits.—We conducted analyses using dichotomized phenotypes. Individuals with BMI $\geq$30 or %fat $\geq$40 were considered to be affected, and individuals with BMI <27 or %fat <27 were considered to be unaffected. The threshold of BMI $\geq$30 was chosen because the majority of people in this category are overfat, not merely overweight (Bray 1978). This BMI threshold was also used in the family-ascertainment design (Price et al. 1998). The %fat thresholds were selected to give numbers of affected and unaffected individuals that were similar to those obtained from the dichotomization of BMI. In the discrete analyses, individuals with intermediate values of BMI (27–30) and %fat (27–40) were considered unknown. Although some information may be lost through dichotomization, we have shown that the majority of variance in our sample is among obese individuals rather than between obese and lean individuals (Price et al. 1998). Discrete phenotypes are desirable because they permit affecteds-only parametric analyses.

Genotyping

We initially genotyped 354 microsatellite markers spaced, on average, 10.1 cM apart; 290 markers were genotyped by means of ABI 373 sequencers (Applied Biosystems), and 64 markers were manually genotyped to reduce intervals >20 cM in the original set. The average heterozygosity for the 354 markers used in the first-stage genome scan was .75. After the first-stage genome search, we observed that 20q markers supported linkage, across most methods and phenotypes. We then saturated this region with 16 additional markers to further localize the linkage results (25 markers total). The 20q markers were, on average, 3.5 cM apart (range 0.01–12 cM).

Framework Genotyping

DNA amplification was performed by means of a PTC100 thermocycler (MJ Research). The forward primer of each pair was labeled with one of three fluorescent dyes—FAM, HEX, or TET (Applied Biosystems)—to enable detection. PCR was performed in a final reaction volume of 20 μl. Amplification occurred during 35 cycles, each for 30 s at 94°C, 30 s at the primer-specific annealing temperature, and 30 s at 72°C. PCR products were subjected to electrophoresis on an ABI373 sequencer using a 12-cm well-to-read denaturing 6% polyacrylamide gel, with analysis by Applied Biosystems' GeneScan 1.2.

Saturation Genotyping and Shortening of Marker Intervals

PCR was conducted in a 10-μl reaction volume under conditions appropriate for the particular marker and

with modifications of protocols described elsewhere (Reed et al. 1995). Alleles were scored by two readers blind to phenotype and were checked for Mendelian incompatibilities by means of the computer program GENEHUNTER. Any incompatibilities were identified, and the original films were reevaluated for scoring or data-entry errors. Markers were rerun as required. For the 20q saturation mapping, we assumed that any apparent double recombinants were typing errors, and we either resolved these through retyping or considered the genotypes to be unknown. In addition, all apparent single recombinants in 20q were examined and verified or were retyped if necessary.

Statistical Analysis

Quantitative-trait phenotypes.—We computed two statistics as implemented by MAPMAKER/SIBS version 2.0 (Kruglyak et al. 1996): the EM algorithm–based Haseman-Elston regression (EMHE) (Haseman and Elston 1972) and the nonparametric-linkage (NPL) quantitative-trait–loci analysis (NPL_QTL) (Kruglyak and Lander 1995). In contrast to the traditional Haseman-Elston regression, the EMHE utilizes information from the full identity by descent (IBD) distribution when computing the regression between IBD and the squared phenotypic difference. The NPL_QTL employs the Wilcoxon rank-sum test to evaluate the relation between the rank of absolute phenotypic difference and IBD at a given locus. This approach is robust to assumptions of both normality of squared phenotypic difference and lack of correlation between the differences and their residuals. We used all sibling pairs in the analysis, and multiple sibling pairs within families were weighted by $\Sigma(N - 1) - 2$, as suggested by Wilson and Elston (1993). The use of phenotypes of all sibling pairs guards against false positives resulting from violations of the assumed underlying distribution (Kruglyak and Lander 1995).

Discrete phenotypes.—We performed single- and multipoint NPL analyses, as implemented by GENEHUNTER version 1.1 (Kruglyak et al. 1996). GENEHUNTER does not require assumptions related to mode of inheritance and penetrance, and it examines whether the allele sharing among affected relatives is greater than expected under the null hypothesis. A greater weight is given to families with a larger number of affected relatives. We applied a weighting function of $1/\sqrt{q}$ in summing the NPL scores, where q equals the number of families. Information content was computed as described by Kruglyak et al. (1996). After the first- and second-stage genome scans, two additional analyses were conducted with the saturation marker set: (1) an affecteds-only model using the FASTLINK version of the linkage program MLINK (Lathrop and Lalouel 1984; Lathrop et al. 1984; Cottingham et al. 1993; Schaffer et al. 1994) and (2) the affected-sibling-pair (ASP) analysis of Terwilliger (SIBPAIR) (Knapp et al. 1994; Kuokkanen et al. 1996). SIBPAIR uses the ASP mean test and incorporates both affected and unaffected siblings to compute a LOD score while assuming an autosomal recessive mode of inheritance. This program weights multiple affected sibships by applying a weight factor, $2/N$, where N is the number of affected siblings. N affected siblings in a sibship contribute $N - 1$ pairs (Suarez and Van Eevdenegh 1984; Blackwelder and Elston 1985). A simulation study by Davis and Weeks (1997) has shown SIBPAIR to be a consistently powerful method across different models.

Allele frequencies.—For all analyses, we assumed a susceptibility-gene frequency of .01 and used marker-allele frequencies based on data on all individuals who provided DNA. This approach gives asymptotically unbiased estimates of the allele frequencies, thereby reducing the risk of false positives (Ott 1992). Allele frequencies were estimated separately for the framework and saturation samples. For a subset of analyses, we conducted separate analyses on Caucasian and African American families, using race-specific allele frequencies to control for possible differences between the two groups.

Genetic Map

To estimate locus order and intermarker distance, we used the map distances from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (WI) (Dib et al. 1996). For markers not found in the WI database, we consulted The Cooperative Human Linkage Database (Sheffield et al. 1995), The Genetic Location Database (Collins et al. 1996), and The Genome Database. Because locus order for some of the markers for the chromosome 20 saturation map was equivocal among different databases, we used the locus order suggested by results from MultiMap (Matise et al. 1994) analyses of our own data.

Simulations

Because we conducted multiple analyses of obesity phenotypes for multiple markers, we completed a simulation study to estimate the genomewide, empirical P values for single-point linkage analyses. Simulations were conducted by means of the computer program SIMULATE (Terwilliger and Ott 1994); this program assumes that there is no linkage between a trait and markers. Using the 92 nuclear families from our study, we simulated markers having five alleles with equal frequency (heterozygosity .80) and placed 10 cM apart. Thus, all markers were simulated to be unlinked to the trait, but the adjacent markers were linked at a distance of 10 cM.

To estimate the genomewide *P* values, we simulated 100 replicates for 35 sets of 10 linked markers, thereby creating 35,000 replicates, roughly simulating linkage groups spanning the genome. We then conducted six analyses of each of the replicates. Four different phenotypes were used: BMI ⩾30 (NPL statistic), BMI as a quantitative trait (EMHE and NPL_QTL statistics), %fat ⩾40 (NPL statistic), and %fat as a quantitative trait (EMHE and NPL_QTL statistics). Thus, we completed a total of 210,000 analyses assuming the same parameters, except for allele frequencies, as those that were used in analyses of the real data. To estimate analysis-specific (single test) *P* values, we computed the empirical *P* values by dividing the number of replicates that exceeded the observed nominal *P* value for that particular test by the total number of replicates (35,000 tests of 35,000 replicates). To control for multiple testing, we computed empirical *P* values by dividing the number of replicates that exceeded the observed *P* value for any of the six tests (210,000 total) by the total number of replicates (35,000).

Results

Subjects

In the first stage of the genome scan, we studied 92 nuclear families from 91 kindreds having 513 individuals (table 1). The overall mean BMI was 35.2 (SD 10.9), and the overall mean %fat was 41.3 (SD 10.8). For BMI, there were 487 sibling pairs, and 144 individual siblings had BMI ⩾30. For %fat, there were 306 sibling pairs, and 87 individual siblings had %fat ⩾40. In the second stage, we included 32 additional families, for a total of 124 nuclear families with 713 individuals. There were an additional 163 sibling pairs for BMI and an additional 117 sibling pairs for %fat. The mean BMI (34.1, vs. 35.2) and %fat (39.1, vs. 41.3) for the 32 additional families did not differ significantly from those in the 92 families initially used. The proportions of siblings exceeding the thresholds for BMI ⩾30 and %fat ⩾40 were somewhat lower in the 32 additional families than in the initial 92 families (36.5% vs. 59.8% for BMI and 20.2% vs. 36.8% for %fat). Although the proportion of African Americans in the 32 additional families was significantly higher than that in the initial sample (32.3% vs. 10.5%; $\chi^2 = 54.5$; $P < .01$), neither the mean BMI (35.7 kg/m^2 vs. 35.9 kg/m^2) nor the mean %fat (40.5 vs. 40.8) differed significantly between Caucasians and African Americans.

Simulations

The single-test empirical *P* values based on simulations did not differ substantially from the nominal *P* values provided by the linkage-analysis programs. However, multiple testing resulted in inflation of *P* values, particularly for $P = .05$. When we controlled for multiple tests, nominal *P* values of .05 and .01 corresponded to empirical *P* values of .189 and .022, respectively, and empirical *P* values of .05 and .01 corresponded to nominal *P* values of .0185 and .0055, respectively. Specific single- and multiple-test empirical *P* values are given for particular markers in tables 2 and 3 and are discussed below.

Table 1

Characteristics of Families in the First and Second Stages of the Genome Scan

	First-Stage Scan	Additional for Second-Stage Scan
No. of families	92	32
No. of individuals	513	200
No. of sibling pairs with measured BMI values	487	163
Mean BMI (SD)	35.2 (10.9)	34.1 (10.4)
No. of sibling pairs with measured %fat values	306	117
Mean %fat (SD)	41.3 (10.8)	39.1 (12.2)
No (%) of siblings with BMI ⩾30	144 (59.8)	38 (36.5)
No. (%) of siblings with %fat ⩾40	87 (36.8)	21 (20.2)
% African American families	10.8	32.3

Single-Point Analyses

First-stage genome scan.—Table 2 summarizes results of the single-point analyses of the 354 framework markers from the initial genome scan. Nineteen analyses of 18 markers from 12 chromosomes had multiple-test empirical *P* values ⩽.05, which is equivalent to 5.1% of markers (18 of 354) and 0.9% of tests (19 of 2,144). There was clustering of linkages on chromosomes 20 and 10. The positive chromosome 20 markers spanned 43 cM of the q arm and gave positive results for three of four obesity phenotypes and for several analytic models. Two of the four markers, D20S200 and D20S107, had multiple-test, empirical *P* values <.01, and the remaining two markers, D20S476 and D20S149, had multiple-test, empirical *P* values close to .01. Positive results for chromosome 10 markers were limited to the EMHE analyses, primarily for BMI, and spanned a large interval of 83 cM. Only one other marker (D2S439) from the initial scan had a multiple-test empirical *P* value <.01 for the EMHE analysis of %fat.

Second-stage mapping of chromosome 20q.—From the first-stage scan, evidence for linkage appeared most strong and consistent for markers from chromosome 20q, across different analytical methods and different phenotypes. We thus added 16 additional markers to 20q, for a total of 25 markers, and completed single-point and multipoint analyses in an augmented sample

Table 2

Summary of Single-Point Analyses of All Markers from Initial Genome Scan (354 Total) with Multiple-Test Empirical *P* Values of .05 for the Six Phenotype × Analysis Combinations

				P Value		
					Empirical	
Chromosome and Marker	Location (cM)	Phenotype	Statistic	Nominal	Single Test	Multiple Test
1:						
D1S194	206	%Fat	EMHE	.0126	.0105	.0297
2:						
D2S439	257	%Fat	EMHE	.0031	.0015	.0045
3:						
D3S1286	37	%Fat	EMHE	.0059	.0036	.0112
7:						
D7S817	43	BMI	EMHE	.0116	.0090	.0265
8:						
D8S560	46	%Fat	EMHE	.0106	.0085	.0239
9:						
D9S1863	151	BMI	EMHE	.0185	.0171	.0499
10:						
D10S1211	87	BMI	EMHE	.0081	.0060	.0166
D10S537	94	BMI	EMHE	.0074	.0053	.0148
D10S670	145	BMI	EMHE	.0109	.0085	.0248
D10S587	170	%Fat	EMHE	.0113	.0090	.0256
12:						
D12S373	41	BMI	EMHE	.0102	.0077	.0226
13:						
D13S168	50	%Fat	EMHE	.0108	.0087	.0245
16:						
D16S539	118	%Fat	EMHE	.0095	.0072	.0207
17:						
D17S796	16	BMI ⩾30	NPL	.0154	.0047	.0390
20:						
D20S200	56	BMI	EMHE	.0036	.0020	.0056
D20S107	66	BMI	EMHE	.0024	.0011	.0032
D20S476	87	BMI ⩾30	NPL	.0066	.0017	.0126
D20S149	99	%Fat	NPL	.0057	.0003	.0106
D20S149	99	BMI ⩾30	NPL	.0120	.0033	.0279

of 124 nuclear families. Table 3 gives results for 14 analyses of 11 20q markers having multiple-test empirical *P* values <.05. Of those 11 markers, 3 (D20S107 [chromosomal location 66 cM] D20S211 [chromosomal location 91 cM], and D20S149 [chromosomal location 99 cM]) had multiple-test, empirical *P* values <.01. Because of the large amount of computer time required for analyses of the simulation data sets, empirical *P* values were not computed for the remaining analyses; instead, nominal *P* values are reported.

Our subsequent single-point parametric and nonparametric analyses using the MLINK and SIBPAIR programs strongly supported linkage for this region (fig. 1). The results from ASP analysis (by SIBPAIR) for BMI ⩾30 approached the thresholds for "significant" linkage that have been proposed by Lander and Kruglyak (1995).

For BMI ⩾30, the single-point MLINK analysis under the affecteds-only model resulted in LOD scores of 3.06 ($P = .00009$) for D20S476, 3.02 ($P = .0001$) for D20S211, and 1.85 ($P = .00178$) for D20S149, whereas the ASP method gave LOD scores of 3.17 ($P = .000067$), 3.16 ($P = .000069$), and 1.52 ($P = .004058$), respectively (fig. 1). LOD scores were not significant for either D20S902 ($P = .07595$ for MLINK and $P = 0.0819$ for ASP) or D20S102 ($P = .11118$ for MLINK and $P = .100086$ for ASP), which lie in the intervals (D20S467–D20S211 and D20S211–D20S149, respectively) separating the three linked markers. Information content did not vary across the region, suggesting the possibility of multiple linked loci.

For %fat ⩾40, the same three markers gave nominally significant LOD scores, but these scores were not as high as those for BMI ⩾30. The single-point LOD scores from the MLINK analysis for D20S476, D20S211, and D20S149 were 1.86 ($P = .001723$), 1.59 ($P =$

.003391) and 2.04 ($P = .001085$), respectively, whereas the single-point LOD scores from the SIBPAIR analysis were 1.93 ($P = .00146$), 1.18 ($P = .01001$), and 1.89 ($P = .00161$), respectively (fig. 1).

Multipoint Analyses

The information content for markers used in the multipoint analyses exceeded 86% for the 20q region. Figures 2 and 3 summarize the results from our multipoint analyses of the saturation of 20q. In general, these analyses continued to support and strengthen findings from the single-point analyses of BMI and %fat. However, the results were not always consistent across different phenotypes and different analytic methods.

For BMI, the support for linkage was strongest in the BMI ⩾30 analysis (fig. 2). The multipoint GENEHUNTER analysis provided strongest evidence of linkage for D20S476 (NPL = 2.55; $P = .0035$) and D20S120 (NPL = 2.59; $P = .0031$), with a possible third peak for D20S149 (NPL = 2.18; $P = .0104$).

The results from the EMHE analysis of the quantitative phenotype provided, in general, weaker support for linkage than was provided by the BMI ⩾30 analysis. For the EMHE analysis, the strongest evidence for linkage was observed for D20S200 ($P = .0124$), which is centromeric to markers supporting linkage in the other analyses. For the NPL_QTL analysis, the pattern of the peaks resembled the results from the BMI ⩾30 analysis, but the support for linkage was much weaker. For both the EMHE and NPL_QTL statistics, D20S476, D20S211, and D20S149 were not significant at nominal P values of .05. In addition, the NPL_QTL analysis of BMI, adjusted for sex and age (standardized residual), yielded the same two primary peaks as were given by the BMI ⩾30 multipoint analysis. The two peaks, flanking D20S196 and D20S120, showed the strongest support for linkage, and the NPL scores improved slightly compared with the unadjusted NPL_QTL scores of 1.03–1.23 for D20S196 and 0.92–1.13 for D20S120.

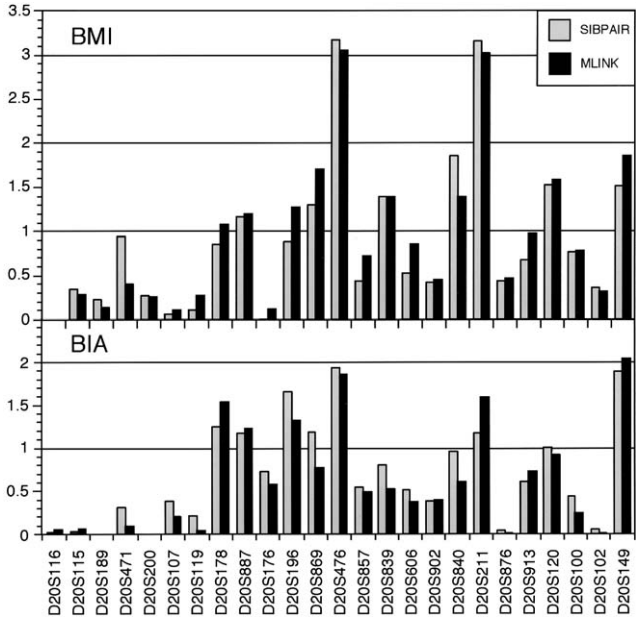

Figure 1 LOD scores for markers from chromosome 20, for parametric single-point analyses implemented by MLINK (using an affecteds-only model) and for nonparametric single-point ASP analyses implemented by SIBPAIR. The upper panel shows the results for BMI ⩾30, and the lower panel shows results for %fat ⩾40.

Table 3

Summary of Single-Point Analyses of Markers from Saturation Mapping of Chromosome 20q (25 Total) with Multiple-Test Empirical *P* Values of .05 for the Six Phenotype × Analysis Combinations

Marker	Location (cM)	Phenotype	Statistic	*P* Value		
					Empirical	
				Nominal	Single Test	Multiple Test
D20S471	47	%Fat	EMHE	.0113	.0090	.0256
D20S200	56	BMI	EMHE	.0098	.0073	.0215
D20S107	66	BMI	EMHE	.0012	.0005	.0014
D20S196	86	BMI ⩾30	NPL	.0154	.0047	.0389
		%Fat ⩾40	NPL	.0062	.0015	.0118
D20S869	86.1	BMI ⩾30	NPL	.0142	.0041	.0349
D20S476	87	BMI ⩾30	NPL	.0182	.0058	.0486
D20S839	88.1	BMI ⩾30	NPL	.0143	.0041	.0351
D20S840	91	BMI ⩾30	NPL	.0163	.0050	.0421
D20S211	91.1	BMI ⩾30	NPL	.0025	.0005	.0034
D20S120	94	BMI ⩾30	NPL	.0104	.0029	.0233
D20S149	99	BMI ⩾30	NPL	.0087	.0022	.0185
		%Fat ⩾40	NPL	.0044	.0011	.0075
		%Fat	NPL	.0032	.0001	.0048

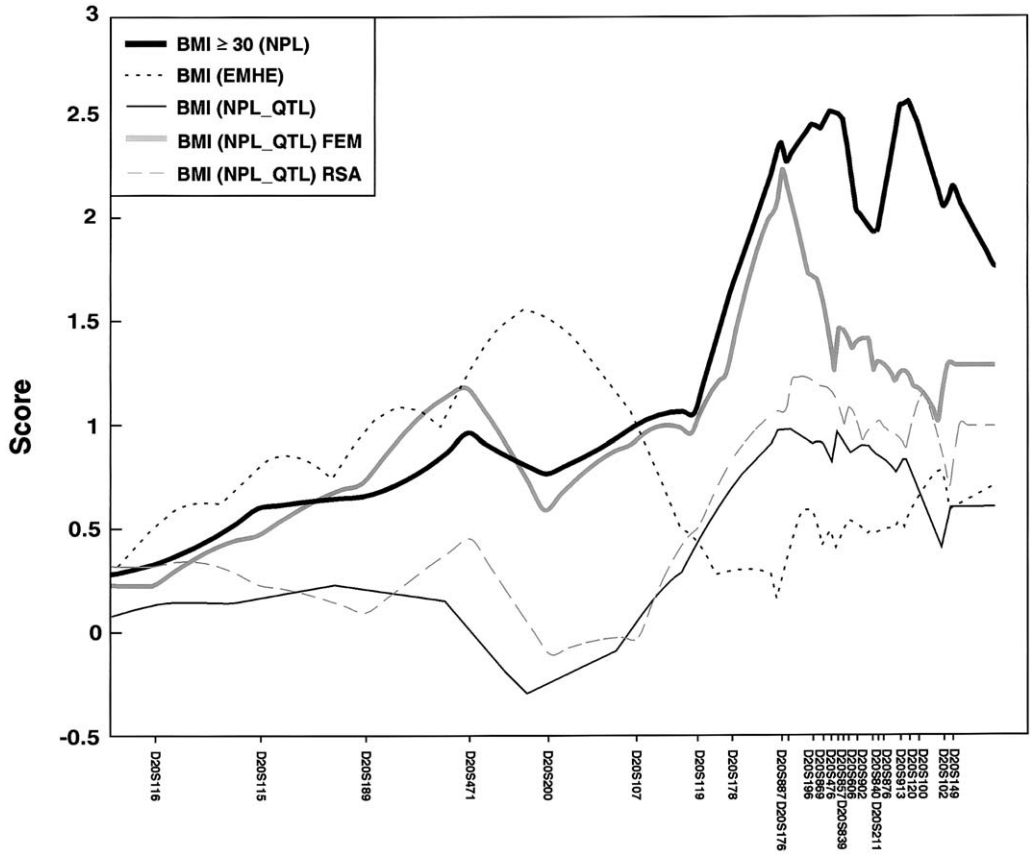

Figure 2 Results of three multipoint methods of analysis for the saturation markers from chromosome 20, with BMI used as the obesity phenotype: the figure key (*upper left*) denotes, in order, NPL analysis implemented by GENEHUNTER, with BMI ⩾30 as a discrete phenotype; EMHE implemented by MAPMAKER/SIBS, with BMI as a quantitative phenotype; NPL statistics implemented by MAPMAKER/SIBS, with BMI as a quantitative phenotype; NPL statistics when only females are used (FEM); and NPL statistics with standardized residuals, after adjustment for sex and age (RSA). Distances between markers are given in centimorgans, and the *y*-axis refers to either the *t* or NPL score for the analyses.

Subsequent, females-only analysis yielded a single peak centering on D20S176, with an NPL score of 2.25 ($P = .0062$).

For %fat, nearly all analyses revealed three peaks, represented by D20S887, D20S120, and D20S149. (fig. 3). For D20S476 and D20S211, the two other markers that were significant in the BMI ⩾30 analysis, the NPL scores were not as significant. D20S476 is ~5.5 cM telomeric to D20S887, and D20S211 is ~3.5 cM centromeric to D20S120; thus, the findings in this set of analyses are similar to those from the BMI ⩾30 analysis, but the locations have been shifted slightly. As with BMI, the results from the adjusted analysis (sex- and age-adjusted residual and females-only analyses) did not differ from those from the unadjusted analyses. In addition, the EMHE method revealed a single peak near D20S471.

Race-Specific Analyses

We restricted our multipoint GENEHUNTER analyses to Caucasians, to control for possible allele-frequency differences between Caucasians and African Americans. The results continued to support the findings from the earlier, unstratified analyses. For BMI ⩾30, three peaks emerged, as before; however, the peaks were slightly more centromeric compared with those from the unstratified analyses. D20S887 (NPL = 2.28; $P = .0079$), D20S839 (NPL = 2.24; $P = .0087$) and D20S913 (NPL = 2.44; $P = .005$) showed the strongest evidence for linkage. However, the results for D20S476 (NPL = 2.2; $P = .010$), D20S120 (NPL = 2.38; $P = .0058$), and D20S149 (NPL = 2.10; $P = .0131$)—which are adjacent to the three markers mentioned

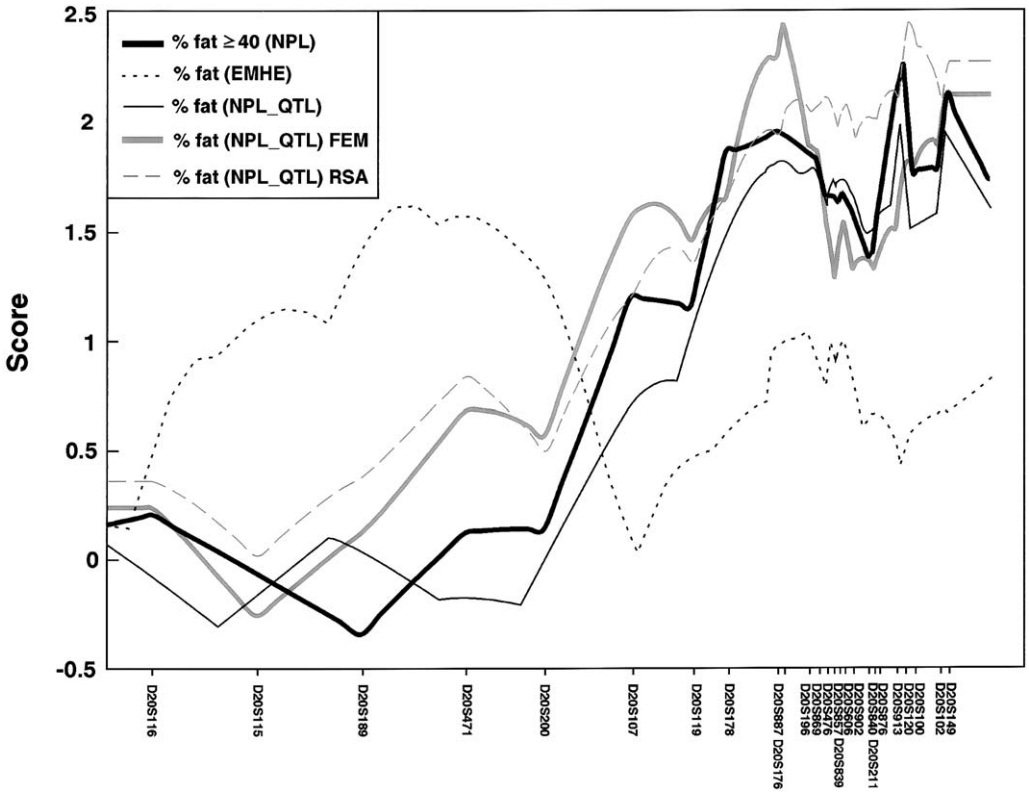

Figure 3 Results of three multipoint methods of analysis, for the saturation markers from chromosome 20, when % body fat as measured by BIA is used as the obesity phenotype: the figure key denotes, in order, NPL analysis implemented by GENEHUNTER, with %fat ⩾40 as a discrete phenotype; EMHE implemented by MAPMAKER/SIBS, with %fat as a quantitative phenotype; NPL statistics implemented by MAPMAKER/SIBS, with %fat as a quantitative phenotype; NPL statistics when only females are used (FEM); and NPL statistics with standardized residuals, after adjustment for sex and age (RSA). Distances between markers are given in centimorgans (cM) and the *y*-axis refers to either the *t* or NPL score for the analyses.

above—were also significant. These markers span ~14 cM (72.0–85.6 cM). For %fat ⩾40, the multipoint GENEHUNTER analysis gave strongest evidence for linkage for D20S178 ($P = .008$), D20S120 ($P = .0056$), and D20S149 ($P = .0063$), and weaker findings were observed for D20S476 (NPL = 1.41; $P = .03480$) and D20S211 (NPL = 1.04; $P = .0896$). There were too few families ($n = 10$) for an African Americans–only analysis.

Discussion

We conducted a genome scan for closely related obesity phenotypes in 92 nuclear families selected for extreme obesity and thinness. Eighteen of 354 markers (19 of 2,124 tests [i.e., 5.1% of markers, 0.9% of tests]) had multiple-test empirical *P* values ⩽.05 in the initial single-point analyses, including quantitative and discrete analyses of BMI and %fat. These positive results included markers on chromosomes 1–3, 7–10, 12, 13, 16, 17, and 20. Five of the 19 positive results were for four markers from chromosome 20q. Given the uncertainties associated with the identification of genes for multigenic traits in humans, we placed relatively greater value on consistency than on any particular *P* value from the initial scan. Of the 12 chromosomes giving positive results with multiple-test empirical *P* values <.05, only chromosome 20 gave consistently positive results across multiple phenotypes, analytic methods, and markers. We selected chromosome 20q for saturation mapping and added an additional 16 20q markers, for an expanded sample of 124 nuclear families.

Single- and multipoint results supported linkage to 20q13; however, the linked interval was large. The discrete results for both BMI and %fat produced three peaks focused on markers D20S476, D20S211, and D20S149, with the results for BMI being somewhat stronger than those for %fat, perhaps because of the larger sample size for the BMI phenotype. The EMHE quantitative analyses gave a more centromeric location

and weaker support for linkage than were given by the other analytic methods.

Additional analyses, controlling for race, sex, and age, gave results that were essentially the same as those from the primary analyses. The insignificant effects of age and gender on linkage analyses of our cohort may be due to (*a*) the extreme nature and early onset of the obesity observed in our sample and (*b*) the fact that most obese family members were female.

When all the analyses were considered, there was some support for linkage over a 16-cM interval from D20S176 to D20S149. However, the strongest support was for the smaller, 10-cM interval in 20q13, containing markers D20S476, D20S211, and D20S149. Markers in the intervals between these markers did not support linkage, suggesting the possibility of multiple linked genes affecting obesity phenotypes in our sample.

Appropriate significance levels for linkage analyses of complex traits are controversial. Diverse approaches have been suggested, ranging from use of nominal *P* values (Curtis 1996; Witte et al. 1996), Bonferroni correction (Wilson et al. 1991; Drigalenko and Elston 1997), simulation-based corrections (Weeks et al. 1990; Norman et al. 1998), to specific critical values (Risch 1991; Lander and Kruglyak 1995; Morton 1998; Rao 1998). Results of our own Monte Carlo simulations suggested a genomewide significance level of approximately $P < .01$ for the 20q13 markers in the initial single-point scan. Multipoint analyses provided additional support and localization of linkage, and the affecteds-only analyses gave nominal *P* values that approach even the most stringent suggested criterion for "significance"—LOD score >3.3 and $P < .00005$ (Lander and Kruglyak 1995). On the basis of a single sample, it is difficult to unambiguously distinguish true from false linkage results. However, linkage of obesity to the chromosome 20q13 region is supported by (1) the consistency of results across related phenotypes and analytic methods, (2) the extremely low *P* values from the sibling pair and parametric analyses, and (3) the results of our simulations.

Additional support for linkage to 20q comes from linkages that other groups have reported for human obesity, mouse obesity, and NIDDM (fig. 4). Borecki et al. (1994) have reported suggestive linkage of BMI and a sum of skin-fold measures to adenosine deaminase. Lembertas et al. (1997) have reported linkage of %fat to markers D20S197 and D20S120, which are close to our markers D20S119 (distance ~5 cM) and D20S476 (distance ~7 cM), respectively. They also found linkage of fat mass and BMI to the MC3R (melanocortin receptor 3) gene, located near D20S149 (≤1 Mb [The Genetic Location Database]). Norman et al. (1998) have reported support for linkage of the 24-h respiratory quotient to markers that are more centromeric (20q11) than the markers found to be significant in our analyses. This

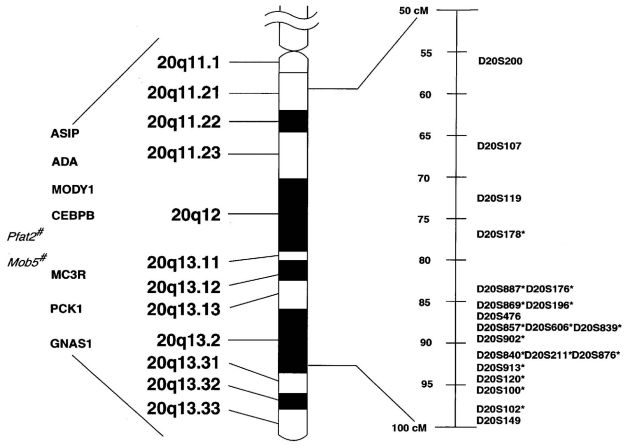

Figure 4 Ideogram of human chromosome 20q. Markers are listed to the right, with their respective chromosomal locations. Markers added for the saturation mapping are denoted by an asterisk (*). The order of polymorphic markers was confirmed by Multi-Map analysis. Human obesity candidate genes are listed to the left. The map order for the human candidate genes was based on databases cited in the text; because of discrepancies among maps, gene order may be different than the best estimate used here. Mouse obesity QTL loci predicted to be homologous to this region are listed to the far left, and denoted by number sign (#). In view of the uncertainty about gene order and intermarker distance in cross-species mapping, these locations are approximate.

region of chromosome 20 also appears to contain two or more genes for diabetes. One form of maturity-onset diabetes of the young (i.e., MODY1) is caused by mutations in the 4HNF4 (hepatic nuclear factor) gene, in 20q12 (Yamagata et al. 1996). Linkage to this region has been reported for NIDDM also (Bowden et al. 1997; Ji et al. 1997). Another NIDDM locus may lie just distal to the interval linked to obesity in our study, near the PCK1 (phosphoenolpyruvate carboxykinase 1) gene (Zouali et al. 1997). Several mouse studies have suggested that genes that influence body weight and fatness in the mouse reside on chromosome 2, in regions homologous to human chromosome 20q (York et al. 1996; Lembertas et al. 1997; Pomp 1997; Taylor and Phillips 1997; Mehrabian et al. 1998). The consistency of the previous human linkage studies for obesity and NIDDM, taken together with the evidence of cross-species homology of obesity genes, supports a role for chromosome 20q in human obesity.

The chromosome region 20q11-q13 contains several genes that are plausible candidates for obesity (fig. 4). Mutations of the agouti gene in mice lead to obesity (Bultman et al. 1992; Miller et al. 1993), and the human orthologue (i.e., agouti-signaling protein [ASIP]) is located within the relevant region of human chromosome 20. ASIP is a potent inhibitor of alpha melanocyte-stimulating–hormone receptors 3 and 4 (MC3R and MC4R)

(Lu et al. 1994; Fong et al. 1997), and mice lacking a functional MC4R gene are obese (Huszar et al. 1997). It is currently unknown whether knockouts of the related MC3R gene would lead to an obese phenotype. Albright hereditary osteodystrophy is a genetic disorder characterized by skeletal and developmental defects and by obesity (Gunay-Aygun et al. 1997). There are several forms of this disorder, and at least two forms are associated with mutations of GNAS1 (guanine nucleotide–binding protein), the gene that maps to human chromosome 20q11-13. Within the linked interval, CEBPB (CAAT/enhancer-binding–protein beta) is another candidate gene, because of its role in adipocyte differentiation (Yeh et al. 1995).

We previously had reported negative linkage for chromosome 20, using a subset of the families that had been collected earlier (Xu et al. 1995). Neither the current nor the previous results supported linkage between D20S476 and D20S149 when BMI and % body fat were analyzed by the Haseman-Elston method. This disparity of results from partially overlapping samples demonstrates that negative reports may be useful only in ruling out gene effects of specific types and sizes, depending on the power associated with the particular sample size and structure and, especially, method of analysis. In our sample, there is a greater range and variability among obese individuals than between obese and normal-weight individuals (Price et al. 1998). Examination of obesity exclusively as a quantitative phenotype, the approach of the earlier report (Xu et al. 1995), may have masked genotype-phenotype relationships, because the phenotype of obese subjects is labile compared with that of normal-weight subjects; discrete thresholds for obesity may be more stable (Reed and Price 1998*a*).

The three human genome–scan studies published to date have implicated three distinct regions for obesity-susceptibility loci (Comuzzie et al. 1997; Norman et al. 1998; present study). Since complex traits are heterogeneous by definition, the absence of complete agreement among genome scans is not surprising. For example, in mouse QTL breeding experiments, starting with different strains of inbred mice yielded different but overlapping sets of loci involved in body-weight regulation (Pomp 1997; Chagnon et al. 1998). In humans, as in the mouse, several genes with different effect sizes could influence obese phenotypes. At least some of our secondary findings should represent true linkages to genes having relatively smaller effects in our sample. Below, we describe positive support for linkage to other chromosome markers, in the context of findings from other studies.

We have observed positive support for linkage in other chromosomes. D7S817 (43 cM) identifies one possible candidate region, although it is located on the p arm, far from known candidate genes or regions in 7q. We previously had reported increased IBD sharing in extremely obese (BMI $\geq$40) sibling pairs, for chromosome 7q31 markers flanking the leptin gene (Reed et al. 1996). The current analyses of less-extreme obesity (BMI $\geq$30) provided little evidence for linkage to this region. However, we continue to find support for linkage between more-extreme obesity phenotypes and the leptin gene region (results not shown). Linkage and association findings for the leptin gene have been mixed, with some studies finding support for linkage (Borecki et al. 1994; Clement et al. 1996; Comings et al. 1996; Duggirala et al. 1996; Reed et al. 1996; Butler et al. 1997; Lapsys et al. 1997; Roth et al. 1997), some finding marginal evidence (Shintani et al. 1996; Oksanen et al. 1997*a*, 1997*b*), and others finding no evidence (Stirling et al. 1995; Bray et al. 1996; Norman et al. 1996, 1997, 1998; Comuzzie et al. 1997; Hasstedt et al. 1997). A recent metanalysis has reported a robust significance level when multiple studies are combined (Allison and Heo 1998). Polymorphisms in the 5′ UTR of the leptin gene that are associated with obesity-related phenotypes have been described (Hager et al. 1998; Mammes et al. 1998), providing further evidence that variability in the leptin gene may influence common forms of obesity in humans.

Marker D1S194 (206 cM) lies in band q21-23, which is homologous with both pig (Andersson et al. 1994) and mouse QTL (Taylor and Phillips 1996). Additionally, a gene for familial partial lipodystrophy, a genetic disorder characterized by loss of subcutaneous adipose tissue, maps to this region (Peters et al. 1998). The positive linkage on chromosome 2 (D2S439; 244.8 cM) is distant from the linkage reported for plasma leptin levels in Mexican Americans (Comuzzie et al. 1997). Marker D3S1286 is near marker D3S2432, for which weak linkage has been reported previously in Pima Indians (Norman et al. 1997). D3S1286 appears to be 37 cM from the homologous region for the mouse obesity locus Dob2 (West et al. 1994) but is near the peroxisome proliferative activated receptor (gamma) (PPARG). The PPARG gene is expressed at high levels in adipoctyes and at very low levels in other tissues and is involved in adipocyte differentiation (e.g., see Shao and Lazar 1997). A polymorphism of this gene is associated with plasma leptin levels in humans (Meirhaeghe et al. 1998).

Marker D8S560 is located near the LPL (lipoprotein lipase) gene, in 8q22. Previous results of linkage and association studies for the LPL gene have been mixed (Comuzzie et al. 1995; Jemaa et al. 1995; Hasstedt et al. 1997). The predominant effect of the mutations in the LPL gene appears to be alterations in lipid metabolism (Mailly et al. 1997); however, one cannot rule out the influence of the genes on body weight. Marker D9S1863 is located in band 9q34. In a previous study of sibling pairs ascertained through hypertensive probands, a marker located in 9q34 gave evidence for link-

age to height, weight, and skin-fold thickness (Wilson et al. 1991). The four positive chromosome 10 markers span 83 cM, which includes most of 10q. Marker D13S168 (50 cM) is near the ESD gene (esterase D), previously linked to obesity phenotypes (Borecki et al. 1994). The linked markers on chromosomes 12 (D12S373; 41 cM), 16 (D16S539; distal 16q), and 17 (D17S796; 16 cM, 17p12) are not near any known candidate genes or regions (Chagnon et al. 1998).

In summary, we have reported strong and significant evidence for linkage between obesity phenotypes and markers in 20q13. We have found consistency across multiple obesity phenotypes, different analytic methods, and several closely linked markers. Both simulation-based and nominal *P* values support the significance of our results. The linkage to markers in a region in 20q13 previously had been supported by both human linkage studies and animal models. This chromosome region should be targeted for further replication, fine mapping, and gene-identification studies.

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Electronic-Database Information

Cooperative Human Linkage Database, The, http://www.chlc.org

Genetic Location Database, The, http://cedar.genetics.soton.ac.uk/public_html/

Genome Database, The, http://gdbwww.gdb.org/

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map/

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