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Interacting Genetic Loci on Chromosomes 20 and 10 Influence Extreme Human Obesity

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Obesity is a multigenic trait that has a substantial genetic component. Animal models confirm a role for gene-gene interactions, and human studies suggest that as much as one-third of the heritable variance may be due to nonadditive gene effects. To evaluate potential epistatic interactions among five regions, on chromosomes 7, 10, and 20, that have previously been linked to obesity phenotypes, we conducted pairwise correlation analyses based on alleles shared identical by descent (IBD) for independent obese affected sibling pairs (ASPs), and we determined family-specific nonparametric linkage (NPL) scores in 244 families. The correlation analyses were also conducted separately, by race, through use of race-specific allele frequencies. Conditional analyses for a qualitative trait (body mass index [BMI] $\geq$27) and hierarchical models for quantitative traits were used to further refine evidence of gene interaction. Both the ASP-specific IBD-sharing probability and the family-specific NPL score revealed that there were strong positive correlations between 10q (88–97 cM) and 20q (65–83 cM), through single-point and multipoint analyses with three obesity thresholds (BMI $\geq$27, $\geq$30, and $\geq$35) across African American and European American samples. Conditional analyses for BMI $\geq$27 found that the LOD score at 20q rises from 1.53 in the baseline analysis to 2.80 (empirical $P = .012$) when families were weighted by evidence for linkage at 10q (D10S1646) through use of zero-one weights ($weight_{0\text{-}1}$) and to 3.32 (empirical $P < .001$) when proportional weights ($weight_{prop}$) were used. For percentage fat mass, variance-component analysis based on a two-locus epistatic model yielded significant evidence for interaction between 20q (75 cM) and the chromosome 10 centromere (LOD = 1.74; $P = .024$), compared with a two-locus additive model (LOD = 0.90). The results from multiple methods and correlated phenotypes are consistent in suggesting that epistatic interactions between loci in these regions play a role in extreme human obesity.

Introduction

Obesity (MIM 601665) is an increasingly prevalent condition associated with adverse consequences for health and quality of life. Comorbid disorders include type 2 diabetes mellitus, hypertension, cardiovascular disease, and some cancers (Carroll 1998; Kopelman 2000; Price 2002; Shmulewitz et al. 2001; Wolk et al. 2001). Family studies demonstrate that obesity and thinness follow family lines (Price 1987; Maes et al. 1997; Price et al. 2000), and twin and adoption studies indicate that most family variance is genetic in origin (Stunkard et al. 1986, 1990; Price 1987; Sorensen et al. 1989; Grilo and Pogue-Geile 1991; Price and Gottesman 1991; Maes et al. 1997).

Gene-gene interactions may be common. Family studies estimate the genetic heritability of obesity at ~40%, and twin studies place the figure higher, at ~65% (Price 2002). The consistent differences in these estimates suggest that as much as one-third of the heritable variance may be due to nonadditive genetic variance, including allelic (dominance and recessivity) and nonallelic gene interactions.

There are some specific examples of known gene interactions in obesity. For example, the extent of obesity and diabetes resulting from a single gene mutant depends on genomic background (Coleman and Hummel 1975). Other animal models provide further support for a role for gene-gene interactions (Ollmann et al. 1997; Niswender et al. 2001). Gene-gene interactions have been reported for several human disorders as well. Linkage studies suggest a possible interaction between the gene *calpain-10* and an unknown gene on chromosome 15, in type 2 diabetes and obesity (Cox et al. 1999; Horikawa et al. 2000). Molecular studies demonstrate that some genes are needed to mediate the phenotypic effects of others (e.g., neuropeptide Y mediates the effects of leptin) (Spiegelman and Flier 2001).

The goal of the present article is to evaluate evidence for interaction among five regions, on three chromo-

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somes, for which evidence of linkage to obesity was found in our previous studies (Reed et al. 1996; Lee et al. 1999; Li et al. 1999; Price et al. 2001).

Subjects and Methods

Since the details of family recruitment have been described elsewhere (Price et al. 1998; Lee et al. 1999), we briefly report the methodological approach used in the present study.

Subjects

The families included in these analyses consist of 200 European American families having 542 siblings and 44 African American families having 125 siblings. Of these 244 families, there were 114 families (98 European American and 16 African American) having both parent's DNA, 124 families (100 European American and 24 African American) having one parent's DNA, and 6 families (2 European American and 4 African American) having neither parent's DNA. Sibship size ranged from two to nine in European Americans and from two to eight in African Americans. Most families (220) have two to five siblings and a median sibship size of three. BMI was calculated on the basis of measured height and weight—that is, as weight (in kg)/height (in m^2). In the correlation analyses, the families of affected sibling pairs (ASPs) were included only if genotyping data were available for chromosomes 7, 10, and 20. Three overlapping obesity thresholds—BMI $\geqslant$27, $\geqslant$30, and $\geqslant$35—were used for computation of identity by descent (IBD). For European American families, these three thresholds yield 456 (282 independent), 329 (223 independent), and 194 (152 independent) ASPs, respectively; for the African American families, there were 131 (78 independent), 96 (63 independent), and 64 (45 independent) ASPs. Definition of "independent ASP" follows the Genehunter program manual.

Markers

Thirty-seven markers were selected from five regions, on three chromosomes (7, 10, and 20), where evidence for linkage to obesity-related phenotypes had been found in our previous linkage studies (Reed et al. 1996; Lee et al. 1999; Li et al. 1999; Price et al. 2001). The five regions included 8 markers that span ~8 cM flanking the leptin gene on 7q31 (D7S685, D7S2501, D7S504, D7S1875, −2548, Shintani [Shintani et al. 1996], D7S530, and D7S2452); 12 markers from three regions on chromosome 10, including 13.5 cM on 10p (D10S582, D10S197, D10S193, and D10S208), 8.4 cM on proximal 10q (D10S1646, D10S1647, D10S1685, D10S537, and D10S535), and 6.7 cM on distal 10q (D10S1679, D10S587, and D10S1656); and 17 markers from a 23.1-cM segment on 20q13 (D20S178, D20S887, D20S176, D20S196, D20S869, D20S857, D20S839, D20S606, D20S902, D20S840, D20S211, D20S876, D20S913, D20S120, D20S100, D20S102, and D20S149). The average heterozygosity was ~0.75, and the average information content (multipoint) was 0.94 for chromosome 7 markers, 0.90 for chromosome 10 markers, and 0.94 for chromosome 20 markers.

Genotyping

DNA was extracted from blood or lymphoblastoid cell lines. PCR amplification and gel analysis of radiolabeled or fluorescently labeled microsatellite markers were performed as described elsewhere (Lee et al. 1999; Price et al. 2001). Band patterns were independently scored by two individuals blinded to the phenotypes. All genotypes were checked for Mendelian inheritance by using the program Genehunter (Kruglyak et al. 1996), and errors were resolved by retyping or recoding as "unknown." Any genetically unrelated parents and siblings were excluded, as were all half-siblings.

Map Locations

Genetic maps for markers were taken from the Whitehead Institute/MIT Center for Genome Research. Markers not found in the Whitehead Institute database were placed using the Genetic Location Database and the Genome Database.

ASP-Specific IBD-Sharing Probability, Family-Specific Nonparametric Linkage (NPL) Score, and Permutation Tests

ASP-specific IBD-sharing probabilities and family-specific NPL scores were obtained by using the computer program Genehunter (Kruglyak et al. 1996). IBD-sharing probability for each independent ASP and NPL score for each family were used for correlation analyses implemented in PROC CORR, of SAS, by use of the Pearson method. Because there were a large number of comparisons across regions ($8 \times 12 \times 17$), because single-point and multipoint analyses were conducted, and because there were three correlated phenotypes, Bonferroni correction may be too conservative. For 9,792 comparisons ($8 \times 12 \times 17 \times 2 \times 3$), the nominal P values required are 5.2×10^{-6} for $P = .05$ and 1.0×10^{-6} for $P = .01$. Therefore, a permutation test was used to determine statistical significance. Using the permuted data, we calculated the correlations on the basis of both the observed IBD-sharing probabilities and the NPL scores on chromosomes 7, 10, and 20. To eliminate the dependence in IBD-sharing probability among the ASPs within the pedigree, we permuted the IBD-sharing probability of one chromosome among all independent ASPs, for each pair of chromosomes examined. For NPL-based

correlation, to control the effect of family size, we permuted the NPL score of one chromosome among the families with the same number of ASPs, for each pair of chromosomes examined. Each permutation generates a new data set in which the null hypothesis that there is no correlation between the markers on the two chromosomes is true. The correlation between markers on different chromosomes is then calculated for the permuted data. This procedure was repeated 1,000 times, which gives a distribution of the correlation under the null hypothesis of no interaction between the two chromosomes. Based on a two-sided test, the empirical significance level for each correlation was estimated by the proportion of permutation samples exhibiting a correlation as large or larger in absolute value than the corresponding absolute value of the observed correlation from the original data. This procedure generates the null distribution for each pair of markers.

The statistical-significance estimate based on the empirically derived null distribution does not reflect our having examined many marker pairs in the present study. To make the needed adjustment for multiple comparisons, we focused on the observed maximal correlations from the IBD analyses, to evaluate overall statistical significance. We focused on the IBD analyses, rather than the NPL scores, because the assumptions required are less stringent and are more likely to be met by the data. Specifically, we used the following procedure to obtain a corrected *P* value for the maximal correlation: we selected the maximal correlation score among all possible pairs of regions on chromosomes 7, 10, and 20; then, we permuted the IBD-sharing probability for markers in each of the regions and picked the highest correlation, from the permuted data, among all possible correlations for chromosomes 7, 10, and 20. The significance of the maximal correlations was based on the proportion of times that the observed maximum was equaled or exceeded by the maximum from the permuted data.

Conditional Analysis of a Qualitative Trait (BMI ⩾27)

For markers on chromosome 20, conditional LOD scores were computed using the program GenehunterPlus (Kong and Cox 1997) on the basis of two weighting approaches, $\text{weight}_{0\text{-}1}$ (family weight was 0 if the NPL score at D10S1646/D10S537 was ⩽0; otherwise, it was 1) and $\text{weight}_{\text{prop}}$ (family weight was the observed NPL score if the NPL score at D10S1646/D10S537 was >0; otherwise, it was 0). The conservative χ^2 test—$\chi^2 = 2\ln_{10}(\text{LOD}_{\text{conditional}} - \text{LOD}_{\text{baseline}})$, for 1 df—and the simulation approach (i.e., the random assignment to families of values for $\text{weight}_{0\text{-}1}$ or $\text{weight}_{\text{prop}}$) were used to assess the significance of the increase in LOD score (Cox et al. 1999).

Linkage Analysis of Quantitative Traits

Multilocus quantitative-trait (BMI, percentage fat mass, waist circumference, and waist:hip circumference ratio) linkage analyses were conducted by Haseman-Elston regression, as implemented in the computer program MapMaker/Sibs (Kruglyak and Lander 1995), and by variance-component analysis using the computer program Solar (Almasy and Blangero 1998). After controlling for the linear effects of age within sex and race categories, we used standardized residuals of obesity phenotypes for Haseman-Elston regression analyses using MapMaker/Sibs. In variance-component analyses, age, sex, and race were adjusted as covariates for the obesity phenotypes, and the ascertainment scheme was accounted for by the identification of primary probands through use of Solar. The following serial hierarchical models were used to examine interaction: (1) two separate one-locus models with marker effects due to each marker locus individually, (2) a two-locus model with marker effects for two loci simultaneously but with no interaction term, and (3) a two-locus epistatic model wherein both marker effects have interaction terms.

Table 1

Four Regions on Chromosomes 10 and 20 with Linkage to Obesity Phenotypes in the Combined Sample

Chromosome	No. of Markers	Map Distances (cM)		Loci		Program	Analysis (Statistic)[a]	Score	Phenotype(s)[b]	Position (cM)
		Start	End	Start	End					
10p	4	46.70	60.30	D10S582	D10S208	Genehunter	NPL (*Z*)	2.68	BMI ⩾27	50.50
10 centromere	5	88.70	97.00	D10S1646	D10S535	Solar	VC (LOD)	2.50	Waist, QTL	88.70
						Solar	VC (LOD)	1.46	BMI, QTL	88.70
						MapMaker/Sibs	EMHE (*t*)	2.24	BMI, QTL	88.70
10q	3	151.60	158.30	D10S1679	D10S1656	MapMaker/Sibs	NPL (*Z*)	2.22	WHR, QTL	156.70
20q	17	64.00	87.06	D20S178	D20S149	Genehunter	NPL (*Z*)	2.25	BMI⩾27	64.50
						MapMaker/Sibs	NPL (*Z*)	2.57	%fat, QTL	75.00

[a] VC = variance component; EMHE = expectation-maximization Haseman-Elston.
[b] Waist = waist circumference; WHR = waist:hip circumference ratio; %fat = percentage fat mass.

Table 2

Correlation between Loci on Chromosomes 10 and 20 on the Basis of ASP-Specific IBD-Sharing Probability

Analysis and BMI	African American Results				European American Results				Combined Results			
	N	Markers (Distance [in cM])	*r*	*P*	*N*	Markers (Distance [in cM])	*r*	*P*	*N*	Markers (Distance [in cM])	*r*	*P*
Single point:												
⩾27	78	D10S582 (46.70)/D20S102 (83.26)	.329	.003	282	D10S582 (46.70)/D20S902 (74.36)	.198	.001	360	D10S582 (46.70)/D20S102 (83.26)	.194	<.001
		D10S537 (94.10)/D20S149 (87.06)	.304	.004		D10S582 (46.70)/D20S839 (72.40)	.190	<.001		D10S582 (46.70)/D20S149 (87.06)	.188	<.001
		D10S1647 (91.50)/D20S196 (69.40)	.299	.006		D10S537 (94.10)/D20S211 (75.36)	.184	.001		D10S1646 (88.70)/D20S876 (71.40)	.187	<.001
		D10S1647 (91.50)/D20S211 (75.36)	.299	.010		D10S582 (46.70)/D20S100 (82.26)	.166	.004		D10S537 (94.10)/D20S211 (75.36)	.187	<.001
		D10S582 (46.70)/D20S887 (64.50)	.298	.009		D10S582 (46.70)/D20S857 (71.40)	.162	.007		D10S582 (46.70)/D20S196 (69.40)	.183	<.001
⩾30	63	D10S1647 (91.50)/D20S196 (69.40)	.377	.001	223	D10S582 (46.70)/D20S902 (74.36)	.224	.001	286	D10S582 (46.70)/D20S149 (87.06)	.239	<.001
		D10S582 (46.70)/D20S102 (83.26)	.377	.001		D10S582 (46.70)/D20S839 (72.40)	.216	<.001		D10S1646 (88.70)/D20S196 (69.40)	.221	<.001
		D10S1646 (88.70)/D20S196 (69.40)	.374	.002		D10S582 (46.70)/D20S606 (70.40)	.196	.005		D10S582 (46.70)/D20S902 (74.36)	.221	<.001
		D10S1647 (91.50)/D20S887 (64.50)	.363	.001		D10S582 (46.70)/D20S100 (82.26)	.196	.003		D10S537 (94.10)/D20S606 (70.40)	.219	<.001
		D10S582 (46.70)/D20S149 (87.06)	.363	.002		D10S537 (94.10)/D20S606 (70.40)	.194	.002		D10S1646 (88.70)/D20S887 (64.50)	.216	.001
⩾35	45	D10S1647 (91.50)/D20S211 (75.36)	.430	.001	152	D10S582 (46.70)/D20S839 (72.40)	.266	.004	197	D10S582 (46.70)/D20S149 (87.06)	.269	.001
		D10S1646 (88.70)/D20S196 (69.40)	.409	.006		D10S582 (46.70)/D20S857 (71.40)	.232	.001		D10S582 (46.70)/D20S102 (83.26)	.241	<.001
		D10S582 (46.70)/D20S149 (87.06)	.408	.003		D10S582 (46.70)/D20S149 (87.06)	.216	.007		D10S1646 (88.70)/D20S196 (69.40)	.230	<.001
		D10S1646 (88.70)/D20S211 (75.36)	.394	.005		D10S535 (97.00)/D20S211 (75.36)	.214	.004		D10S582 (46.70)/D20S196 (69.40)	.226	.002
		D10S1647 (91.50)/D20S902 (74.36)	.376	.011		D10S537 (94.10)/D20S606 (70.40)	.212	.006		D10S537 (94.10)/D20S606 (70.40)	.213	<.001
Multipoint:												
⩾27	78	D10S1646 (88.70)/D20S149 (87.06)	.371	<.001	282	D10S197 (50.60)/D20S100 (82.26)	.205	<.001	360	D10S582 (46.70)/D20S149 (87.06)	.200	<.001
		D10S1646 (88.70)/D20S876 (71.40)	.354	<.001		D10S197 (50.60)/D20S102 (83.26)	.194	<.001		D10S582 (46.70)/D20S102 (83.26)	.196	<.001
		D10S1647 (91.50)/D20S149 (87.06)	.351	<.001		D10S582 (46.70)/D20S102 (83.26)	.191	<.001		D10S582 (46.70)/D20S100 (82.26)	.195	<.001
		D10S1646 (88.70)/D20S102 (83.26)	.343	.001		D10S582 (46.70)/D20S100 (82.26)	.189	<.001		D10S1646 (88.70)/D20S913 (78.36)	.182	.002
		D10S1646 (88.70)/D20S913 (78.36)	.337	.001		D10S193 (59.10)/D20S100 (82.26)	.178	.001		D10S1646 (88.70)/D20S100 (82.26)	.177	.001
⩾30	63	D10S1646 (88.70)/D20S149 (87.06)	.418	.001	223	D10S197 (50.60)/D20S100 (82.26)	.222	<.001	286	D10S582 (46.70)/D20S102 (83.26)	.236	<.001
		D10S1646 (88.70)/D20S102 (83.26)	.403	.001		D10S582 (46.70)/D20S102 (83.26)	.218	<.001		D10S582 (46.70)/D20S100 (82.26)	.233	<.001
		D10S1646 (88.70)/D20S887 (64.50)	.399	.002		D10S582 (46.70)/D20S100 (82.26)	.216	<.001		D10S582 (46.70)/D20S149 (87.06)	.230	<.001
		D10S1647 (91.50)/D20S149 (87.06)	.397	.001		D10S197 (50.60)/D20S102 (83.26)	.206	.001		D10S1646 (88.70)/D20S887 (64.50)	.218	<.001
		D10S1646 (88.70)/D20S100 (82.26)	.393	.002		D10S193 (59.10)/D20S100 (82.26)	.195	.001		D10S1646 (88.70)/D20S178 (64.00)	.214	<.001
⩾35	45	D10S1646 (88.70)/D20S102 (83.26)	.481	<.001	152	D10S197 (50.60)/D20S100 (82.26)	.227	.004	197	D10S1646 (88.70)/D20S178 (64.00)	.268	<.001
		D10S1646 (88.70)/D20S100 (82.26)	.473	<.001		D10S1646 (88.70)/D20S178 (64.00)	.225	.003		D10S1646 (88.70)/D20S887 (64.50)	.250	.002
		D10S1646 (88.70)/D20S887 (64.50)	.435	.001		D10S582 (46.70)/D20S102 (83.26)	.220	.008		D10S582 (46.70)/D20S149 (87.06)	.239	.001
		D10S1646 (88.70)/D20S120 (79.36)	.435	.002		D10S197 (50.60)/D20S102 (83.26)	.219	.003		D10S1646 (88.70)/D20S869 (68.40)	.239	.002
		D10S1646 (88.70)/D20S606 (70.40)	.429	.001		D10S582 (46.70)/D20S100 (82.26)	.215	.010		D10S582 (46.70)/D20S102 (83.26)	.231	.002

Results

Baseline Multipoint Linkage Analyses

In the combined sample, multipoint linkage analyses indicated that there were five regions, on chromosomes 7, 10, and 20, that had at least marginal evidence for linkage to obesity phenotypes. Table 1 presents the four regions, on chromosomes 10 and 20, that have been linked to obesity-related phenotypes. Markers on 10p (51 cM) and 20q (65 cM) had evidence for linkage with BMI ⩾27, with an NPL score of $Z > 2.2$. Markers on the chromosome 10 centromere were linked with quantitative traits, namely BMI and waist circumference. Markers on 10q (157 cM) and 20q (75 cM) were linked with waist:hip circumference ratio and percentage fat mass, respectively. These findings, based on a larger sample than that used in the original studies, are similar to results from our previous studies (Reed et al. 1996; Lee et al. 1999; Li et al. 1999; Price et al. 2001).

Correlation Analyses Based on ASP-Specific IBD-Sharing Probability

All of the pairwise correlations between markers on chromosome 7 and markers on chromosomes 10 and 20 approached 0.0. Table 2 includes the five highest correlation scores between chromosomes 10 and 20 on the basis of IBD-sharing probability of independent ASPs by race, obesity threshold, and analytic approach. For the African American sample, most of the observed highest correlations occurred within 5 cM (89–94 cM) of D10S1646 and 7 cM (69–83 cM) of D20S211. The rest were found between D10S582 and 20q (65–87 cM). The highest correlation based on multipoint analysis was found at D10S1646 (89 cM) and 20q (83–87 cM) through three thresholds. For the European American sample, however, most observed highest correlations occurred within 5 cM (46–51 cM) of D10S197 and 20q (72–83 cM). The rest were found between 10q (89–97 cM) and 20q (64–75 cM). The highest cor-

Table 3

Correlation between Loci on Chromosomes 10 and 20 on the Basis of Family-Specific NPL Score

Analysis and BMI	Markers (Distance [in cM])	Correlation Score (Empirical *P*)		
		African Americans (N = 43)	European Americans (N = 167)	Combined (N = 210)
Single point:				
⩾27	D10S1647 (91.50)/D20S887 (64.50)	.342 (.012)	.339 (.003)	.344 (.002)
	D10S1647 (91.50)/D20S196 (69.40)	.362 (.008)	.334 (.014)	.340 (.008)
	D10S1646 (88.70)/D20S606 (70.40)	.171 (.142)	.329 (<.001)	.299 (.009)
	D10S1685 (94.00)/D20S211 (75.36)	.241 (.061)	.313 (.002)	.297 (.002)
	D10S537 (94.10)/D20S211 (75.36)	.135 (.202)	.334 (<.001)	.296 (.004)
	D10S1685 (94.00)/D20S196 (69.40)	.434 (.001)	.242 (.040)	.285 (.021)
	D10S535 (97.00)/D20S211 (75.36)	.094 (.276)	.328 (.002)	.282 (.020)
⩾30	D10S537 (94.10)/D20S606 (70.40)	.348 (.013)	.293 (<.001)	.302 (<.001)
	D10S1646 (88.70)/D20S887 (64.50)	.421 (<.001)	.218 (.027)	.273 (<.001)
	D10S1646 (88.70)/D20S196 (69.40)	.535 (<.001)	.180 (.087)	.245 (.001)
⩾35	D10S537 (94.10)/D20S606 (70.40)	.188 (.113)	.354 (.001)	.329 (<.001)
	D10S537 (94.10)/D20S211 (75.36)	.253 (.051)	.335 (.006)	.323 (.003)
	D10S535 (97.00)/D20S211 (75.36)	.060 (.348)	.361 (.002)	.311 (.002)
	D10S1646 (88.70)/D20S211 (75.36)	.437 (.001)	.277 (.035)	.310 (.003)
	D10S1646 (88.70)/D20S196 (69.40)	.542 (<.001)	.197 (.016)	.266 (.005)
Multipoint:				
⩾27	D10S535 (97.00)/D20S839 (72.40)	.281 (.033)	.270 (.001)	.273 (.005)
	D10S1685 (94.00)/D20S176 (67.40)	.352 (.011)	.248 (.002)	.271 (.009)
	D10S535 (97.00)/D20S902 (74.36)	.283 (.035)	.264 (.001)	.267 (.009)
	D10S1685 (94.00)/D20S196 (69.40)	.336 (.014)	.248 (.001)	.265 (.010)
	D10S1685 (94.00)/D20S869 (68.40)	.341 (.014)	.245 (.002)	.263 (.013)
	D10S1646 (88.70)/D20S839 (72.40)	.195 (.106)	.282 (<.001)	.260 (.006)
⩾30	D10S1646 (88.70)/D20S606 (70.40)	.334 (.017)	.168 (.015)	.207 (.007)
	D10S1646 (88.70)/D20S211 (75.36)	.252 (.050)	.177 (.010)	.198 (.006)
⩾35	D10S1646 (88.70)/D20S211 (75.36)	.352 (.011)	.302 (<.001)	.311 (.002)
	D10S1646 (88.70)/D20S876 (77.36)	.402 (.004)	.281 (.001)	.304 (.002)
	D10S1646 (88.70)/D20S120 (79.36)	.408 (.004)	.281 (<.001)	.304 (.001)
	D10S1646 (88.70)/D20S913 (78.36)	.410 (.004)	.276 (.001)	.302 (.001)
	D10S1646 (88.70)/D20S840 (74.86)	.335 (.013)	.284 (<.001)	.293 (.002)
	D10S1646 (88.70)/D20S606 (70.40)	.429 (.002)	.259 (.001)	.287 (.002)
	D10S1646 (88.70)/D20S902 (74.36)	.391 (.005)	.241 (.002)	.269 (.005)

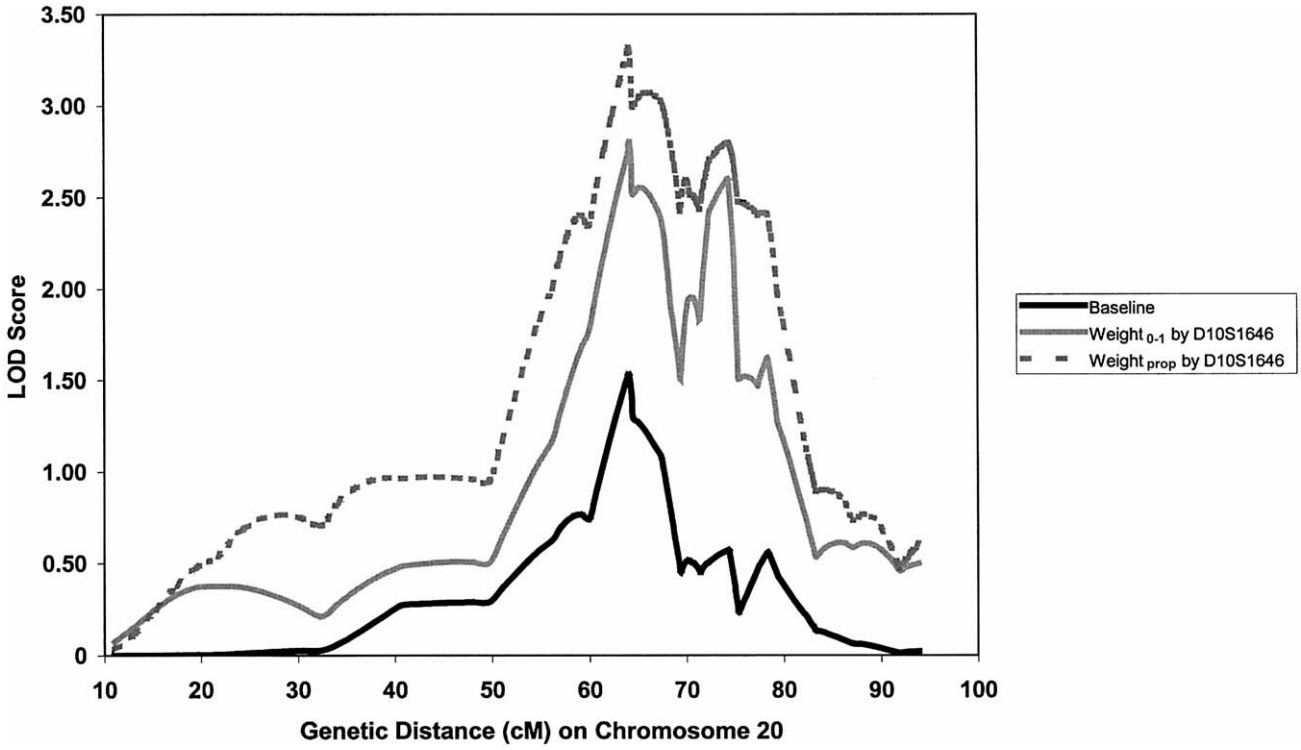

Figure 1 Multipoint analyses of chromosome 20 for BMI ⩾27 in the combined sample. Conditional allele-sharing multipoint analyses were performed by weighting the families on the basis of the evidence for linkage at D10S1646.

relation by multipoint analysis was found between D10S197 and D20S100 through three thresholds. For the combined sample, the locations of the observed highest correlations were shifted slightly on chromosomes 10 and 20. One occurred between D10S582 and 20q (82–87 cM), and another occurred between D10S1646 and 20q (64–68 cM).

When we used the permutation procedure to account for multiple comparisons in the multipoint analyses, the corrected *P* values for maximal correlation score were .019 (correlation 0.200), .007 (correlation 0.236), and .018 (correlation 0.268), for BMI ⩾27, ⩾30, and ⩾35, respectively. As expected, correction for multiple testing reduced the level of significance by as much as an order of magnitude, but the correlations remained significant, with $P < .02$.

Correlation Analyses Based on Family-Specific NPL Score

Table 3 presents correlation scores based on family-specific NPL score, with empirical $P < .01$ in at least one of the three samples. All the correlations were localized within 10q (89–97 cM) and 20q (65–75 cM). Most observed correlations occurred across both subgroups. For the African American sample, the highest correlation was found between D10S1646 and 20q (69–70 cM) for single (0.542 [empirical $P < .001$]) and multipoint (0.429 [empirical $P = .002$]) analyses. For the European American sample, the highest correlation was 0.361 (empirical $P = .002$) for single-point analysis and 0.302 (empirical $P < .001$) for multipoint analysis. For the combined sample, the maximal correlation of 0.311 (empirical $P = .002$) was found between D10S1646 and D20S211 (210 families with BMI ⩾35). The *P* values (corrected for multiple testing) for the maximal correlation score from the NPL analyses were .013 (correlation 0.273), .065 (correlation 0.207), and .002 (correlation 0.311), for BMI ⩾27, ⩾30, and ⩾35, respectively.

Conditional and Epistatic Interaction Analyses

Figures 1 and 2 show the LOD scores based on the combined sample for the baseline and conditional multipoint allele-sharing analyses (BMI ⩾27) of chromosome 20, weighted by the evidence for linkage at D10S537 and D10S1646, respectively. Compared with baseline analyses, conditional analyses had the largest increment in LOD score at 20q (75 cM) for both weighting approaches. The LOD score at the peak rose from 1.53 in the baseline analysis to 2.80 ($\chi^2 = 5.85$; nominal $P = .016$; empirical $P = .012$) when families were weighted by evidence for linkage at 10q (D10S1646)

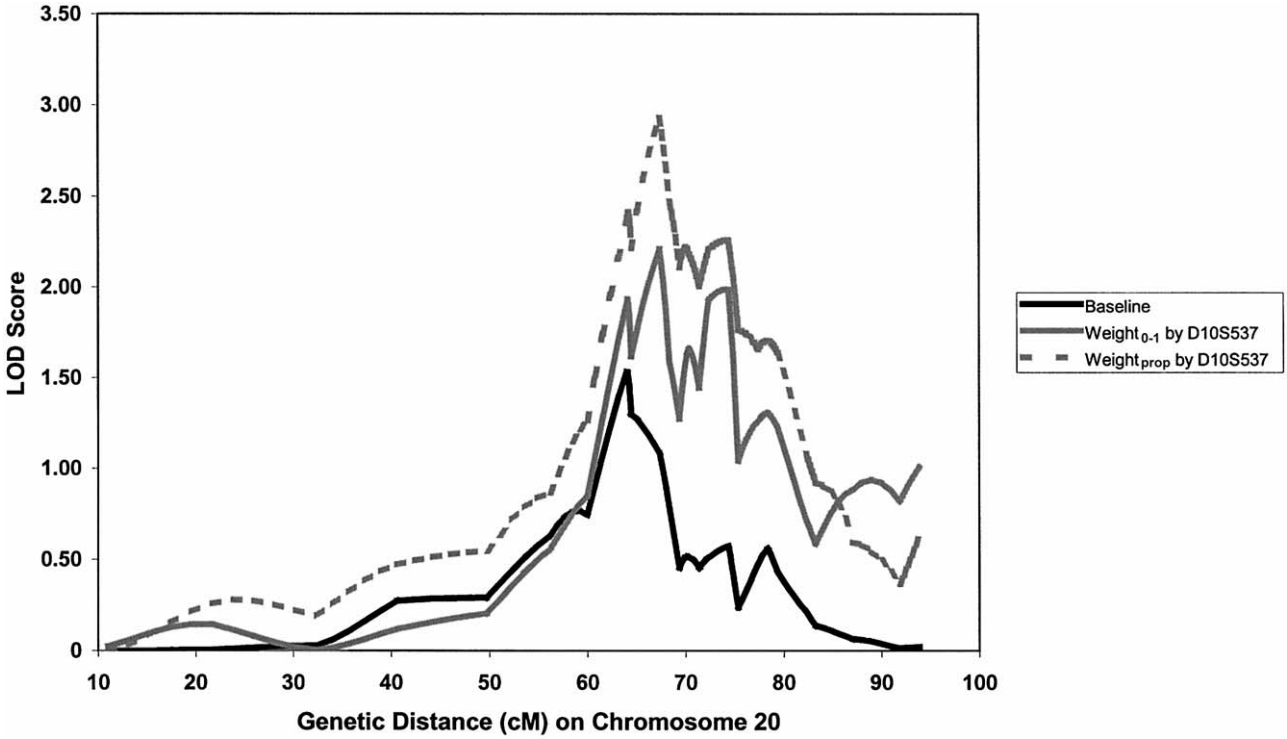

Figure 2 Multipoint analyses of chromosome 20 for BMI ⩾27 in the combined sample. Conditional allele-sharing multipoint analyses were performed by weighting the families on the basis of the evidence for linkage at D10S537.

through use of $\text{weight}_{0\text{-}1}$ and to 3.32 ($\chi^2 = 8.24$; nominal $P = .004$; empirical $P < .001$) when $\text{weight}_{\text{prop}}$ was used (fig. 1). Similarly, the LOD score at the peak rose to 2.21 ($\chi^2 = 3.13$; nominal $P = .077$; empirical $P = .040$) when families were weighted by evidence for linkage at 10q (D10S537) through use of $\text{weight}_{0\text{-}1}$ and to 2.93 ($\chi^2 = 6.45$; nominal $P = .011$; empirical $P = .007$) when $\text{weight}_{\text{prop}}$ was used (fig. 2). For quantitative traits, oligogenic analyses were performed using both additive and epistatic two-locus models, to look for evidence of joint effects. Figure 3 shows that there was a significant epistatic effect on percentage fat mass, between 20q (75 cM) and proximal 10p (LOD = 1.74; $P = .024$), compared with a two-locus additive model (LOD = 0.90). The P value obtained from the likelihood-ratio test of an epistatic two-locus model was compared with the two-locus additive model. No significant epistatic effects on other quantitative phenotypes were found in these regions.

Discussion

Obesity has a substantial heritable component and has been found to be associated with or linked to >250 genomic regions (Perusse et al. 2001; Rankinen et al. 2002), but the identification of genes responsible for common forms of obesity continues to be difficult. Heritability studies suggest that nonadditive gene effects could account for as much as one-third of all genetic variation in obesity. However, to date, few studies have attempted to incorporate gene interaction in obesity-linkage analyses.

Since the mode of inheritance is largely unknown and is likely to be complex, the present study evaluated the evidence for gene interaction primarily by using nonparametric methods. We calculated correlations, in ASP-specific IBD-sharing probabilities and family-specific NPL scores, between unlinked regions on chromosomes 7, 10, and 20. Since we did not find any positive correlation, with nominal $P < .01$, between these regions and the chromosome 7 loci that have been linked to obesity phenotypes, the present study focused detailed analyses on chromosomes 10 and 20. Based on single-point and multipoint analyses and thresholds, the observed correlation scores revealed that there is an interaction between genes on 10q and on 20q. This conclusion was also supported by results from conditional analyses and hierarchical models, by using a variance-component approach.

The major challenge for gene-gene–interaction detection is that a potentially large number of comparisons is possible. We have adopted strategies to control for overall type I error rates while increasing the likelihood that possible gene-gene interactions will be iden-

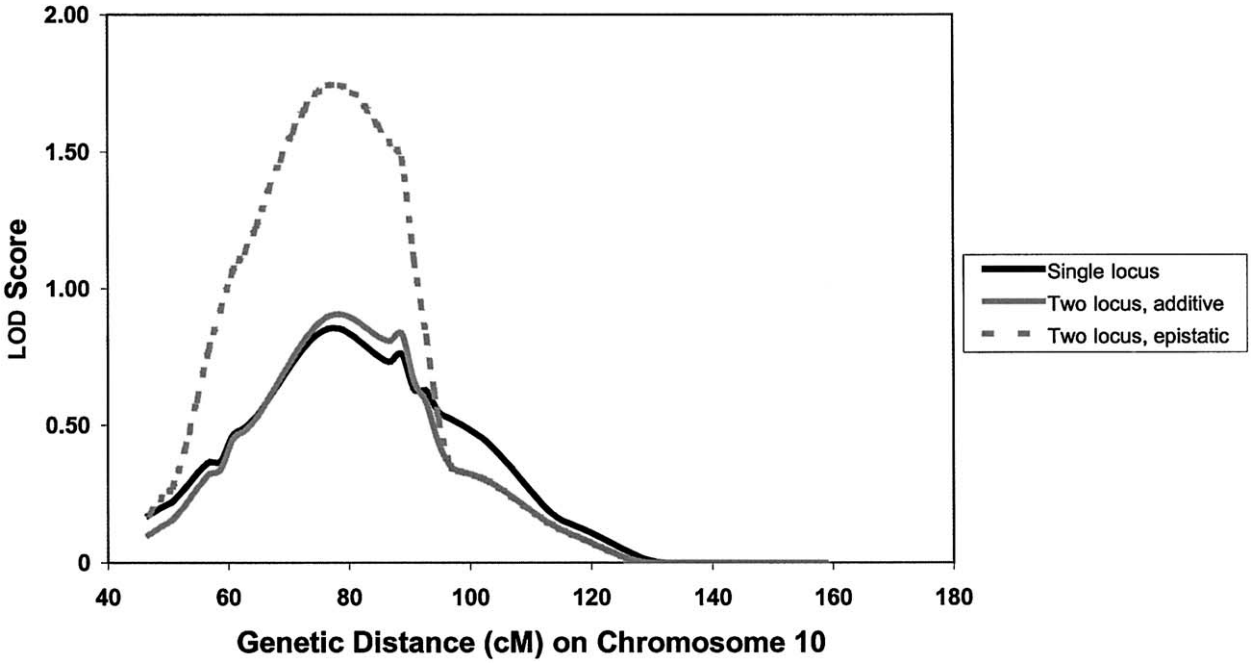

Figure 3 Epistatic-interaction analyses for percentage fat mass by variance-component approach in the combined sample. Oligogenic analyses were performed using both additive and epistatic two-locus models to look for evidence of joint effects.

tified. Accordingly, the overall statistical significance was assessed through simulation studies. Our major findings for correlations in IBD-sharing probabilities were significant even after correction for multiple testing, through permutation analyses. The relative simplicity of the IBD correlational approach requires few assumptions, and they should be met by many data sets. For this reason, we believe it should be preferred over correlations in NPL scores.

To date, several independent linkage studies have suggested the existence of obesity-predisposition loci on 20q (Norman et al. 1998; Lee et al. 1999; Hunt et al. 2001; Perusse et al. 2001; Deng et al. 2002; Rankinen et al. 2002). The reported regions appear to be too broad for study differences to be due to poor gene localization. The breadth of the linked interval, as well as the appearance of multiple peaks, suggests that there are multiple susceptibility genes in this region. In fact, 20q is rich in genes involved in signaling and contains several putative candidate genes for obesity. ASIP (agouti-signaling protein) is a protein inhibitor of MC3R and MC4R (melanocortin receptors 3 and 4, respectively) (Fong et al. 1997); it has been reported that inactivation of MC3R results in increased body fat at the expense of lean body mass (Chen et al. 2000) and that mutations of *ASIP* lead to obesity in mice (Miller et al. 1993). CEBPB (CAAT/enhancer-binding protein β) is related to adipocyte differentiation (Yeh et al. 1995). Mutations of GNAS1 (guanine nucleotide-binding protein α-stimulating activity polypeptide 1) were associated with Albright hereditary osteodystrophy partly characterized by obesity (Gunay-Aygun et al. 1997). It is noteworthy that conditional analyses based on evidence of linkage at either D10S1646 or D10S537, spanning 10 cM on 20q, resulted in similar increased evidence for linkage. This finding may reflect poor localization of the linkage signal; however, it may also indicate a complex interaction among common polymorphisms at different loci involved in common expression pathways that differ in frequency among families.

Although French and German studies and our study of the present cohort found markers on 10p to be linked to obesity (Hager et al. 1998; Hinney et al. 2000; Price et al. 2001), candidate genes for obesity within the identified regions are not well characterized. A French study suggested that there is a major gene locus, on 10p (D10S197; maximum LOD score 4.85), that is implicated in the development of human obesity. It is very interesting that, in the correlation analyses based on ASP-specific IBD-sharing probability, D10S197 (peak-NPL-score locus in the French study and the present study cohort) and D10S582 (~3 cM from D10S197) gave a strong correlation with markers on 20q in the European American sample only. However, the strongest correlation in family-specific NPL score was found between proximal 10q markers and 20q13 markers across both subgroups in the present study. The French study also found a secondary linkage peak at the 10q centromeric location. Both the conditional analyses for a qualitative trait (BMI $\geq$27) and the hierarchical model for a

quantitative trait (percentage fat mass) revealed that there was an epistatic interaction between the two regions. Proximal 10q appears to be somewhat richer in genes than 10p is, but the association between these genes and obesity is largely unknown. Because the observed interactions in the present study are at a statistical (rather than biological) level, independent study will be needed to confirm and further characterize these interactions.

Conclusion

The present study provides evidence that genes in proximal 10q and 20q may interact to increase susceptibility to human obesity. More attention should be paid to epistatic gene-interaction effects in the identification of genes for this complex disorder. Caution should also be used in the interpretation of linkage evidence in the presence of interaction. Independent replication in multiple studies remains critical for the identification of genes controlling complex traits.

Acknowledgments

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Electronic-Database Information

The accession number and URLs for data presented herein are as follows:

Genetic Location Database, The, http://cedar.genetics.soton.ac.uk/public_html/ldb.html (for map location)
Genome Database, The, http://gdbwww.gdb.org/ (for map location)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for obesity [MIM 601665])
Whitehead Institute/MIT Center for Genome Research, http://www-genome.wi.mit.edu/ (for map location)

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