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Interaction between obesity-susceptibility loci in chromosome regions 2p25-p24 and 13q13-q21

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One of the chief complexities of genetic influences on human obesity appears to be gene–gene interactions. Here, we employed model-free approaches to look for gene–gene interaction effects in human obesity using genome scan data from 260 European American families. We found consistent evidence for statistical interaction between 2p25-p24 (18–38 cM) and 13q13-q21 (26–47 cM). For discrete traits, the positive correlations were significant at $P < 0.0001$ ($P \leq 0.0023$ after correction for multiple tests) in both IBD-based and NPL-based analyses for BMI ≥ 40 kg/m². Other analytic approaches gave consistent, supportive results. For quantitative traits, interaction effects were significant for BMI ($P = 0.0012$), percent fat ($P = 0.0265$) and waist circumference ($P = 0.0023$) in a Haseman–Elston regression model, and for BMI ($P = 0.0043$) in variance component analysis. Our findings suggest that obesity-susceptibility loci in chromosome regions 2p25-p24 and 13q13-21 may interact to influence extreme human obesity. The identification of gene–gene interactions may prove crucial to understanding the contributions of genes, which, by themselves, have relatively small effects on obesity susceptibility and resistance.

European Journal of Human Genetics (2005) 13, 102–108. doi:10.1038/sj.ejhg.5201292

Published online 6 October 2004

Keywords: linkage; correlation; regression; variance components; interaction; obesity

Introduction

Obesity is one of the top public health problems in the world and is associated with increased risk for type II diabetes mellitus, hypertension, cardiovascular diseases and some cancers.^{1–5} Family, twin and adoption studies of human obesity have demonstrated that a substantial portion of family variance is genetic in origin, with up to one-third of the genetic variance due to nonadditive factors.^{6–14} Many single genes have been examined through association studies, and multiple quantitative trait loci have been identified through genome scans. However, only a few, mostly very rare, monogenetic forms of human obesity have been detected.¹⁵ The pattern of inheritance of obesity and the rarity of mutations in known major genes

strongly suggest a multigenic inheritance with individual gene effects mediated by genomic background and environmental influences.

A number of linkage studies, including ~30 human genome scans, have reported about 70 genetic loci linked with obesity in humans.¹⁵ The results vary substantially across studies and ‘replications’ (reported linkages in the same region) have been limited, including 2p22, 3q27, 6p21, 10p12, 11q23-11q24, 17p12 and 18q21. A common aspect of these studies is that most were based on small samples, and only a small number of results reached a genome-wide level of significance. Apart from these issues of power and significance, the generally weak results may be due to the overly simplified models of inheritance we and others have employed.

Several lines of evidence suggest epistatic effects on obesity may be common. Consistent differences in estimates of genetic heritability of obesity between family and twin studies indicate that about one-third of the heritable variance may be nonadditive.¹⁶ Several animal models give

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Received 28 April 2004; revised 27 July 2004; accepted 13 August 2004

clear evidence of gene–gene interactions.^{17–19} Molecular genetic studies also demonstrate that some genes are needed to mediate the effects of others.²⁰

Studies of several complex traits have identified susceptibility loci by allowing for gene–gene interaction in the linkage analyses, including diabetes,²¹ asthma,²² psoriasis²³ and systemic lupus erythematosus.²⁴ We have reported a potential epistatic effect between chromosome 10 and 20q in the linkage analysis similar to those employed in the current paper.¹⁴

In the present study, we extended our analyses to look for potential interacting genetic loci among several regions linked to obesity phenotypes based on our recently completed second-generation genome-wide linkage study in 260 European American families segregating extreme obesity and normal weight.²⁵

Subjects and methods

Families

This study included 260 European American families ascertained nationwide in a genetic study of obesity at the University of Pennsylvania. The recruitment processes have been described previously.^{26,27} After obtaining informed consent, the probands and their biological parents and full siblings were measured for weight, height, waist circumference, percent body fat and other anthropometric measurements. Blood samples were also collected. Details about the characteristics of this sample have been recently reported elsewhere.²⁵

Phenotypes

For qualitative traits, we used four overlapping dichotomies of obesity affection status based on measured BMI (BMI \geq 27, 30, 35 and 40 kg/m²), which was computed by dividing the measured weight in kilograms by the square of measured height in meters (kg/m²). The four thresholds yield 658 (401 independent in 224 sibships), 472 (316 independent in 203 sibships), 282 (215 independent in 163 sibships) and 134 (115 independent in 100 sibships) affected sib pairs, respectively. We defined independent affected sib pairs according to the definition in the GENEHUNTER manual by taking the first affected sib paired with other affected sibs (2nd, 3rd, ...*n*th affected sib) in the sibship. For analyses of quantitative traits, we used measured BMI, percent fat and waist circumference after controlling the linear effects of age within sex and generation.

Genotyping

A total of 382 polymorphic microsatellite markers were genotyped by Marshfield Center for Medical Genetics from the Version 11 Weber Screening Set, which has an average marker spacing of 10 cm. Genetic map distances were taken from the Marshfield Database (<http://research.marshfield->

[clinic.org/genetics](http://research.marshfield-)). All genotypes were checked for Mendelian inheritance using the computer program MERLIN with the ‘–error’ option (v0.9)²⁸ and incompatible genotypes were recoded as unknown.

Genome-wide baseline linkage analyses

The primary methods used for our second-generation genome scan data have been presented recently.²⁵ In brief, we used the GENEHUNTER program to perform nonparametric multipoint linkage analyses using four overlapped qualitative phenotypes.²⁹ Allele frequencies were computed based on all individuals who provided DNA. We then employed the MERLIN computer program to conduct regression-based quantitative-trait linkage analyses using the standardized residuals of BMI, percent fat (%fat) and waist circumference after linear correction for age within generation and sex.

Epistatic interaction analyses of qualitative traits

Two approaches were used to screen for potential epistatic effects on discrete obesity phenotypes.³⁰ The first examined correlations between IBD-sharing proportions or linkage statistics (eg NPL score) at unlinked loci among affected sib pairs (ASPs) or families. We calculated pairwise Pearson’s correlation coefficients for ASP-specific IBD-sharing proportion and family-specific NPL score between unlinked loci selected according to our genome-wide linkage analyses. We computed the multipoint ASP-specific IBD-sharing proportion and family-specific NPL score using GENEHUNTER.

We conducted permutation tests to calculate empirical *P*-values. To eliminate the potential dependence in IBD-sharing probability among the ASPs within a single family and control the effect of family size, we permuted the data among independent ASPs for IBD correlation analyses and among the families with the same number of ASPs for NPL correlation analyses. We generated 10 000 replicates by permutation. For each replicate, we computed the correlation coefficients among all possible pairwise loci and recorded the maximum correlation score over all the pairwise correlations. We then calculated corrected *P*-value to adjust for multiple comparisons based on the proportion of times that the observed correlation score was equaled or exceeded by the maximal correlation score of each replicate. Details about the application of a permutation test to family and sibling data have been previously reported elsewhere.¹⁴

The second approach used was conditional linkage analyses as proposed by Cox *et al.*²¹ We performed conditional analysis only in the regions demonstrating evidence for positive correlations at a corrected *P* < 0.05. In the conditional analyses, we used zero-one and proportional weighting schemes to model a positive relationship between loci on different chromosomes. We computed baseline (unconditional) and conditional LOD (logarithm

of odds) scores using the ALLEGRO computer program (v1.2c).³¹ We assessed the significance of the increase in LOD score on the basis of a conservative χ^2 test, as proposed by Cox *et al.*²¹

Epistatic interaction analyses of quantitative traits

We used two popular approaches, Haseman–Elston (H–E) regression model and variance component analysis, to detect epistatic interactions using quantitative phenotypes of obesity.

We used the new H–E regression model by incorporating epistatic interactions between the loci.^{30,32,33}

In this model, the mean-corrected trait product for a sib pair is regressed on the underlying IBD-sharing proportions at different loci as predictors. To account for the dependencies among multiple sib pairs created from the same family, we incorporated the method of generalized estimating equations (GEE) into H–E regression analyses using GENMOD procedures in SAS (v8.2).

In variance components analyses, the phenotypic covariance between relatives was modeled in terms of variance component parameters and underlying IBD-sharing probabilities at one or more genetic loci. We examined epistatic interactions by comparing the likelihood of the models with and without epistatic components of variance. We used age, sex and generation as covariates for the obesity quantitative phenotypes and accounted for the ascertainment scheme by the identification of the primary probands. We performed the analyses using the SOLAR computer program (v1.7).^{34,35}

Results

Loci with linkage evidence in genomewide scan

Table 1 lists the loci with an NPL score ≥ 1.85 and the significance at $P < 0.025$ (GENEHUNTER output P) for a discrete BMI phenotype (BMI $\geq 27, 30, 35$ and 40 kg/m^2) using GENEHUNTER or with an LOD score ≥ 1.44 (MERLIN output $P \leq 0.005$) for a quantitative obesity phenotype

Table 1 Loci with multipoint NPL scores ≥ 1.85 or LOD ≥ 1.44

Chromosome	Position (cM)	Marker	Score	P	Analysis (statistics) ^a	Phenotype ^b
2	0	SRA	1.46	0.0050	REG (LOD)	Waist, QTL
	38	D2S1360	1.70	0.0030	REG (LOD)	BMI, QTL
3	124	D3S3045	1.88	0.0178	NPL (Z_all)	BMI $\geq 30 \text{ kg/m}^2$
	188	D3S2427	1.50	0.0050	REG (LOD)	BMI, QTL
4	143	D4S1644	1.71	0.0020	REG (LOD)	BMI, QTL
7	109	D7S821	1.93	0.0077	NPL (Z_all)	BMI $\geq 35 \text{ kg/m}^2$
	114	D7S1799	2.25	0.0024	NPL (Z_all)	BMI $\geq 35 \text{ kg/m}^2$
8	94	D8S2324	1.90	0.0083	NPL (Z_all)	BMI $\geq 35 \text{ kg/m}^2$
9	104	D9S910	2.09	0.0010	NPL (Z_all)	BMI $\geq 30 \text{ kg/m}^2$
12	18	GATA49D12	2.12	0.0128	NPL (Z_all)	BMI $\geq 27 \text{ kg/m}^2$
	109	PAH	1.92	0.0151	NPL (Z_all)	BMI $\geq 30 \text{ kg/m}^2$
13	125	D12S2070	3.79	< 0.0001	REG (LOD)	%fat, QTL
	26	D13S1493	2.03	0.0215	NPL (Z_all)	BMI $\geq 40 \text{ kg/m}^2$
	33	D13S894	2.63	0.0043	NPL (Z_all)	BMI $\geq 40 \text{ kg/m}^2$
	47	D13S1807	2.67	0.0002	REG (LOD)	BMI, QTL
	55	D13S800	2.70	0.0002	REG (LOD)	BMI, QTL
	76	D13S793	2.78	0.0002	REG (LOD)	BMI, QTL
	83	D13S779	2.82	0.0002	REG (LOD)	BMI, QTL
21	58	D21S1446	4.27	< 0.0001	REG (LOD)	%fat, QTL

^aREG: family regression performed in MERLIN; NPL: nonparametric linkage analysis performed in GENEHUNTER; LOD: logarithm of odds; Z_all: Z statistic in GENEHUNTER.

^bBMI: body mass index; Waist: waist circumference; %fat: percent fat; QTL: quantitative trait locus.

Table 2 Marker pairs with significant ($P \leq 0.05$) correlations after correction for multiple testing

Markers (cM)	R	N^a	Empirical P	Corrected P	Phenotypes	Correlation
D2S1360 (38)/D13S1493 (26)	0.272	161	0.0003	0.0386	BMI35 kg/m^2	NPL
D2S1360 (38)/D13S1493 (26)	0.426	98	0.0000	0.0016	BMI40 kg/m^2	NPL
D2S1360 (38)/D13S1493 (26)	0.410	102	0.0000	0.0023	BMI40 kg/m^2	IBD
D2S1360 (38)/D13S894 (33)	0.357	98	0.0004	0.0251	BMI40 kg/m^2	NPL
D2S1360 (38)/D13S894 (33)	0.341	102	0.0003	0.0347	BMI40 kg/m^2	IBD

^aNumber of families for NPL-based analysis and affected sib pairs for IBD-based analysis.

(BMI, percent fat, waist circumference) using the MERLIN program in our initial analysis of genome scan.²⁵ We used the criteria to include the loci with marginal linkage evidence in the analysis of qualitative or quantitative traits.

IBD and NPL-based correlation analyses

To detect dependence between obesity-linked loci, we computed pairwise correlation between the markers on different chromosomes. Table 2 includes IBD and NPL-based correlation scores between the marker pairs with corrected significance levels of $P < 0.05$. For NPL-based correlation analyses, the observed highest correlations occurred between D2S1360 (38 cM) and D13S1493 (26 cM), with a maximal correlation score of 0.272 (empirical $P = 0.0003$, corrected $P = 0.0386$) and 0.426 (empirical $P < 0.0001$, corrected $P = 0.0016$) for BMI ≥ 35 and ≥ 40 kg/m², respectively. For IBD-sharing proportion-based analyses, the strongest correlation was detected between D2S1360 (38 cM) and D13S1493 (26 cM), with a correlation score of 0.410 (empirical $P < 0.0001$, corrected $P = 0.0023$), whereas the correlations approached zero for both unaffected sib pairs (BMI < 27 kg/m², $N = 71$, $r = 0.044$, $P = 0.7152$) and discordant sib pairs (BMI < 27 kg/m² and BMI ≥ 40 kg/m², $N = 434$, $r = 0.056$, $P = 0.2460$). The strongest and most consistent interactions were for chromosome 2 (18–38 cM) and chromosome 13 (26–47 cM). In the remaining analyses, we focused on this interaction, since the permutation test and associated correction for multiple testing should be most valid for the highest correlation.

Conditional linkage analyses

The results of conditional linkage analyses are summarized in Table 3. The LOD scores at 2p markers (18–38 cM) increased from 0.06 to 2.06 for BMI ≥ 30 kg/m², from 0.07 to 2.12 for BMI ≥ 35 kg/m² (Figure 1a) and from -0.17 to 1.96 for BMI ≥ 40 kg/m² when conditioned on the linkage evidence at D13S1493 (26 cM). The LOD scores at 13q markers (26–33 cM) increased from 0.50 to 2.95 for BMI ≥ 30 kg/m², from 0.85 to 3.11 for BMI ≥ 35 kg/m² and from 1.04 to 4.54 for BMI ≥ 40 kg/m² when conditioned on the linkage evidence at 2p markers (18–38 cM).

H-E multiple-regression and variance component analyses

We further conducted H-E multiple-regression and variance component analyses to examine epistatic effects between 2p markers (18–38 cM) and 13q markers (26–47 cM) using quantitative traits. Table 4 gives the interaction effects for an epistatic model between the two regions with the significance at $P < 0.05$ in H-E regression analyses. The observed interaction effects were significant at $P = 0.0003$ (GEE $P = 0.0012$) for BMI, $P = 0.0136$ (GEE $P = 0.0265$) for %fat, and $P = 0.0012$ (GEE $P = 0.0023$) for waist circumference. Figure 1b shows the LOD scores for markers across chromosome 2 based on a two-locus model

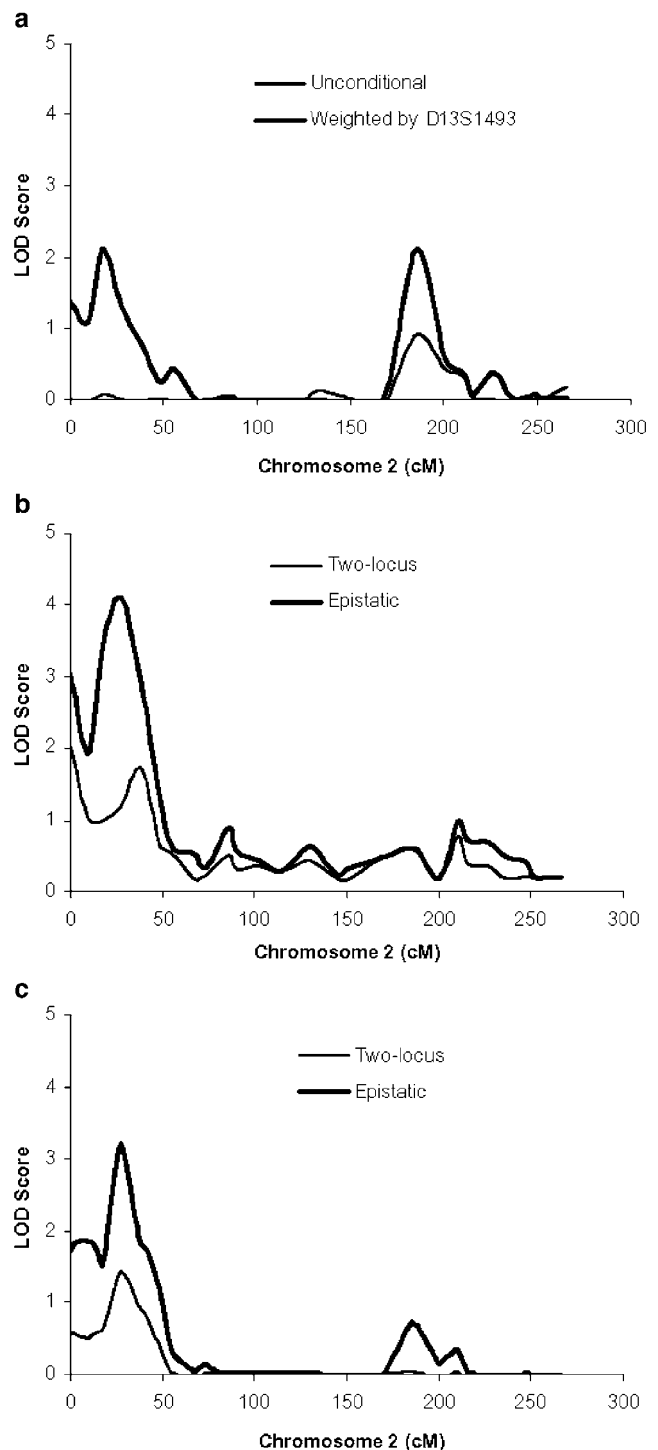


Figure 1 (a) Conditional allele-sharing multipoint analyses of chromosome 2 for BMI ≥ 35 , weighting families based on evidence for linkage at D13S1493 (26 cM). (b) Analysis of epistatic interaction between D13S1493 (26 cM) and markers on chromosome 2 for BMI using an H-E regression model. (c) Analysis of epistatic interaction between D13S1807 (47 cM) and markers on chromosome 2 for BMI using a variance components model.

Table 3 Epistatic effects for discrete phenotypes based on conditional linkage analysis

Detected marker (cM)	Conditional marker (cM)	Phenotype	Baseline LOD	Conditional LOD	Weighting scheme	χ^2	P
D2S2952 (18)	D13S1493 (26)	BMI30 kg/m ²	0.06	1.93	0-1	8.64	0.0033
			0.06	2.06	Prop	9.24	0.0024
D2S2952 (18)	D13S1493 (26)	BMI35 kg/m ²	0.07	2.12	0-1	9.47	0.0021
			0.07	1.89	Prop	8.40	0.0038
D2S1360 (38)	D13S1493 (26)	BMI40 kg/m ²	-0.17	1.00	0-1	5.39	0.0202
			-0.17	1.96	Prop	9.78	0.0018
D13S894 (33)	D2S2952 (18)	BMI30 kg/m ²	0.50	2.95	0-1	11.25	0.0008
			0.50	2.89	Prop	11.00	0.0009
D13S894 (33)	D2S2952 (18)	BMI35 kg/m ²	0.85	3.11	0-1	10.44	0.0012
			0.85	2.60	Prop	8.06	0.0045
D13S1493 (26)	D2S1360 (38)	BMI40 kg/m ²	1.04	4.24	0-1	14.74	0.0001
			1.04	4.54	Prop	16.13	<0.0001

Table 4 Epistatic effects based on H-E regression analyses of quantitative phenotypes

Phenotype	Marker	χ^2	Two-locus model ^a		χ^2	Epistatic model ^b	
			P	GEE P		P	GEE P
BMI (N = 1157)	D2S1400	4.46	0.0347	0.068	3.15	0.0760	0.0905
	D13S1493	0.75	0.3872	0.3534	6.23	0.0125	0.0075
	D2S1400*D13S1493				12.86	0.0003	0.0012
%fat (N = 860)	D2S2952	2.59	0.1072	0.1999	1.21	0.2704	0.3745
	D13S1493	0.21	0.6448	0.6552	3.25	0.0715	0.0988
	D2S2952*D13S1493				6.09	0.0136	0.0265
Waist (N = 996)	D2S2952	1.47	0.2250	0.2888	3.86	0.0495	0.0886
	D13S1493	0.26	0.6082	0.5629	5.64	0.0176	0.0093
	D2S2952*D13S1493				10.46	0.0012	0.0023

^aMultipile-regression did not include interaction term of the markers.

^bMultipile-regression included interaction term of the markers.

(chromosome 2 marker and D13S1493) and an epistatic two-locus model (chromosome 2 marker, D13S1493 and their interaction term) in H-E multiple-regression analysis. Similarly, compared with results from an independent two-locus model, LOD scores for the epistatic model increased for BMI at 2p (28 cM, $P=0.0043$, Figure 1c) when conditioned on 13q marker (47 cM) in variance component analysis.

Discussion

Obesity-susceptibility loci in chromosome regions 2p25-p24 and 13q13-q21 appear to interact in influencing the development of extreme obesity in European Americans. The evidence was consistent across phenotypes and analytic approaches. For discrete traits, correlations based on IBD and NPL scores were significant after correction for multiple tests, whereas correlations between the two regions approached zero for both concordant unaffected sib pairs and discordant sib pairs. Conditional LOD scores

at 13q (26–33 cM), compared with the baseline, increased by more than 2.2 for all obesity thresholds when conditioned on evidence for linkage at 2p (18–38 cM). Analyses of quantitative traits using H-E regression and variance component models also provided strong and consistent support for an interaction.

Multiple testing is a major issue in analyses of gene-gene interactions. In four ways, we attempted to limit untoward effects of the many possible tests in a genome scan while preserving power. First, we limited our focus to regions that reached at least marginal significance in a genome scan. Second, we used permutation tests to estimate empirical significance of individual results. Third, we controlled for multiple testing in the initial correlation analyses. Fourth, we focused extensive analyses only on the strongest and most consistent result from the initial analyses of gene-gene interactions, that between regions of chromosomes 2 and 13.

Several groups have reported linkage in the region of chromosome 2 (2p25-p24) which we detected in our recent genome scan (Li *et al*^{25, 36–38}). There are a very large number

of genes in the 2p25-p24, but a few are plausible candidate genes that previously have been associated with obesity. For example, ACP1 (acid phosphatase 1 gene, 2p25) is a highly polymorphic enzyme involved in the modulation of signal transduction by insulin. Its six genotypes have substantial variation in total enzymatic activity. Linkages and associations of ACP1 with BMI and obesity-related phenotypes have been reported in several independent studies.^{39–42} Another candidate gene, APO-B (apolipoprotein B, 2p24-p23), has been associated with BMI and %fat.^{43–45} POMC (pro-opio-melanocortin) is another obesity-associated gene that lies within 13 cm of our peak marker (D2S1360, 38 cm) on 2p15. Other plausible candidates have been found using animal models, including SDC1 (syndecan-1)⁴⁶ and LIPIN.⁴⁷

Evidence for the presence of linkage between obesity traits and markers on 13q14-21 has been reported in several independent studies. Feitosa *et al*⁴⁸ detected evidence for linkage with BMI on 13q14 marker D13S257 in a combined sample of 401 three-generation families.⁴⁸ A genome-wide scan in the Quebec Family Study also reported a linkage between abdominal subcutaneous fat and D13S325 (47 cm, 13q14).⁴⁹ Knoblauch *et al*⁵⁰ detected evidence for linkage between 13q21 (the second peak on chromosome 13 in our study, Table 1) and BMI ($P < 0.0001$) in a DZ twin sample. One plausible candidate gene from this region of chromosome 13 is 5-hydroxytryptamine (serotonin) receptor gene (5-HTR_{2A}), which is located at 13q14-q21 and has been reported to be associated with obesity. Serotonin is an important mediator in the control of satiety mechanisms. Serotonin reduces food intake and is probably involved in weight regulation.⁵¹ A polymorphism in 5-HT_{2A} receptor gene promoter has been reported to be associated with abdominal obesity.⁵² A polymorphism in 5-HTR_{2A} has also been shown to be associated with dietary energy and alcohol intake in obese people.⁵³

It is possible that several genes in chromosome regions 2p25-24 and 13q13–21, including but not limited to those listed above, could interact to influence the development of obesity. As the observed interactions are at a statistical (rather than biological) level, further and independent studies are needed to confirm and refine the observed results. While it will not be easy or even straightforward to identify the specific interactions at a molecular level, the knowledge that a statistical interaction exists should prove useful and could even turn out to be crucial to the identification of specific gene polymorphisms having relatively subtle effects on obesity phenotypes.^{21,54}

Summary

The present study provides statistical evidence that genetic loci in chromosome regions 2p25-p24 and 13q13-21 may interact to influence the development of human obesity.

Further study and independent replications are needed to confirm and refine the observed results. The identification of gene interactions may be crucial to understand the roles played by specific gene polymorphisms in complex traits such as obesity.

Acknowledgements

This research was supported in part by National Institute of Health grants R01DK44073, R01DK48095 and R01DK56210 to RAP. Genotypes were completed by the NHLBI supported Marshfield Genotyping Service. We gratefully acknowledge the generous cooperation of participating families. We thank Ms Elizabeth Joe, Ms Carmeron Braswell, Ms Quan Cao, Dr Balasahib Shinde, Mr Jeffrey Hannah, Ms Jan Merideth and Ms Kye Yun for technical assistance.

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