

Genome-Wide Scan of Obesity in the Old Order Amish*

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ABSTRACT

To identify the genetic determinants of typical obesity, we performed a genome-wide scan of obesity-related traits using data from the Amish. Multipoint linkage analysis was performed using a variance components procedure on body mass index (BMI), waist circumference, percentage of body fat, and serum leptin concentrations. All 672 individuals were genotyped for 357 markers in 22 autosomes. We observed modest evidence for linkage, with the maximum log odds (lod) scores for linkage for these traits occurring on chromosomes 3p (percentage of body fat: lod = 1.61, near the peroxisome proliferator-activated receptor- α gene), 14q (waist: lod = 1.80), and 16p (leptin:

lod = 1.72; BMI: lod = 1.68). We also tested for linkage to BMI-adjusted leptin concentrations and observed suggestive evidence for linkage on chromosome 10p (lod = 2.73), approximately 10–20 cM telomeric from obesity loci previously reported in French and German Caucasians. Two additional linkage signals for this trait were observed on chromosomes 7q (lod = 1.77, ~20 cM from the leptin gene) and 14q (lod = 2.47). Follow-up studies may be warranted to pursue some of these linkage signals, especially those detected near known obesity candidate genes, and those in regions coinciding with linkage signals reported previously. (*J Clin Endocrinol Metab* 86: 1199–1205, 2001)

OBESITY HAS BECOME a global health issue across all age groups, especially in industrialized countries. In the United States alone, over one third of the population is overweight, defined by having a body mass index (BMI) of 25 kg/m² or greater (1). Obesity is a well-established risk factor for many chronic diseases, such as type 2 diabetes, hypertension, gallbladder disease, osteoarthritis, and some cancers (2). A higher BMI is also associated with higher all-cause mortality (3). The economic cost attributable to obesity is close to \$100 billion/yr (4).

Human obesity is a multifactorial syndrome influenced by genetic, physiological, behavioral, and socio-cultural factors (5). Twin and family studies have shown that genes contribute substantially to variation in body fat accumulation. Research into the genetic determinants of obesity has progressed rapidly in the past decade. Genes responsible for several rare Mendelian forms of obesity have been identified (6), although specific genes contributing to the common form of obesity are largely unknown.

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Segregation analyses suggest that single genes with relatively large effects as well as polygenes with smaller effects contribute to the development of obesity. Studies in several populations suggest that major genes inherited in a recessive manner may account for 35–45% of the variation in obesity-related traits [e.g. BMI, fat mass, and percentage of body fat (percent fat)] after adjusting for the effects of sex and age (7–9). However, other studies have failed to implicate major gene effects, inferring instead a large contribution from multiple genes, each having small effects individually (10–12). Genome-wide linkage studies have been carried out in at least four populations in an attempt to localize obesity susceptibility genes, and chromosomal regions with suggestive linkage have been reported in several populations, including Mexican Americans (13, 14), Pima Indians (15–17), and Caucasians (18, 19). In this paper we report the results of a genome scan of obesity and obesity-related traits conducted in a genetically homogeneous founder population, the Old Order Amish (OOA) in Lancaster County, PA. We also present the results of a simulation study conducted to estimate the power to detect linkage in this unique sample.

Subjects and Methods

Subjects

The OOA is a founder population of European origin. Nearly all of the 30,000 OOA currently alive can trace their lineages back 12–14 generations to some 200 founding couples (20, 21). The OOA are a rural-living population and are characterized by their eschewal of technological innovation and their strong interest in their ancestry and genealogical relationships. Moreover, there is considerable homogeneity in the Amish lifestyle (22).

Recruitment for the Amish Family Diabetes Study began in early 1995 with the goal of identifying genes influencing the risk of type 2 diabetes

and related traits. The study protocol was approved by the institutional review board at the University of Maryland School of Medicine, and informed consent was obtained from each study participant. With the help of liaisons from the OOA community, we identified individuals with type 2 diabetes. These probands and their family members, aged 18 yr and older, were invited to participate. Between February 1995 and February 1997, 691 subjects received examinations at the Amish Research Clinic in Strasburg, PA, or in their homes. Because the recruitment is ongoing, analyses in this paper were restricted to 672 individuals in whom diabetes status was determined and on whom a genome scan was performed. The recruitment strategy and study design have been described in detail previously (22).

Phenotypes

Anthropometric measurements (including BMI, waist circumference, and percent fat) were obtained at the time of clinic or home visit. BMI was calculated as weight (kilograms) divided by height (meters) squared. A measuring tape was used to obtain minimum waist girth at the natural indentation or at the level midway between the iliac crests (and the lower edge of the rib cage if no natural indentation was present) to the nearest 0.5 cm. The percent fat was estimated by bioelectroimpedance using the resistance method (23). A weak radio frequency signal was applied to four electrodes attached to the subject's extremities, thus allowing measurement of the resistances. Sex-specific formulas were then applied to estimate the fat-free mass based on the magnitude of the resistance and body weight. The percent body fat was estimated as total body weight minus fat-free mass, expressed as a proportion of body weight. Serum leptin concentrations were measured from fasting blood samples by RIA (Linco Research, Inc., St. Louis, MO). Its interassay coefficient of variation was 4.25%. Subjects with a BMI of 30 kg/m² or more were defined as obese.

Genotypes

DNA was extracted from leukocytes, and a screening set of 357 highly polymorphic microsatellite short tandem repeat markers was genotyped from the ABI Prism Linkage Mapping Set (PE Applied Biosystems/Perkin-Elmer Corp., Foster City, CA). The mean marker heterozygosity was 0.75 (range, 0.33–0.91). The average intermarker interval was 10.2 cM. The largest gap between markers was 25.4 cM, occurring on chromosome 7. The genotyping error rate based on blind replicates was 0.16%, on the average.

Statistical analysis

Although all subjects can be related by tracing their ancestors back multiple generations, we divided the sample into 28 discrete families to reduce computational burden. These 28 families ranged in size from 3–69 subjects and provided a large number of relative pairs, including 606 parent-offspring pairs, 1460 sibling pairs, 2035 avuncular (aunt/uncle-niece/nephew) pairs, and 1781 first cousin pairs.

Quantitative trait multipoint linkage analysis was carried out using a variance components methodology. We partitioned variation in the obesity phenotype into components attributable to environmental covariates, the additive effects of genes (*i.e.* residual heritability), and a specific quantitative trait locus (QTL; *i.e.* the linkage component). These analyses were conducted using maximum likelihood procedures as implemented in the SOLAR program (24). The residual heritability was modeled as a function of the expected genetic covariances between relatives, and the QTL effect was modeled as a function of the identity by descent relationships at the marker locus. The hypothesis of linkage was evaluated by the likelihood ratio test, which tests whether the locus-specific effect is significantly greater than zero (*i.e.* $H_0: \sigma^2_{QTL} = 0$ vs. $H_A: \sigma^2_{QTL} > 0$). In each model, we simultaneously adjusted for the effects of sex, sex-specific age, and age (2). The total number of individuals included for each trait-specific analysis ranged from 613–662. To reduce the skewness of the phenotype distributions, extreme outliers were excluded from analysis. Leptin levels were transformed by their natural logarithm. We also analyzed obesity as a dichotomous trait, using a cut-point of BMI of 30 kg/m² or greater to classify subjects as obese. For this analysis, we employed an extension of the variance components methodology, which is also implemented in the SOLAR

program. The method is based on a threshold model that assigns an individual a specific affection status if the underlying genetically determined risk or liability exceeds a specific threshold on a normally distributed liability curve (25). The latent liability is assumed to have an underlying multivariate normal distribution with equal unit variances of liability in both the general population and relatives of affected individuals.

After initially obtaining log odds (lod) scores for linkage by the likelihood ratio test, we then evaluated the probability of obtaining false positive results by generating a large number of unlinked markers and evaluating evidence for linkage to these simulated markers. We simulated each unlinked marker locus to have five equally frequent alleles, and then assigned genotypes to all pedigree members based on the genotypes simulated for the founding members of the pedigree. We then conducted linkage analysis of each obesity-related trait with each simulated unlinked marker. The unlinked marker loci were simulated using PAP4 software (26). We simulated a total of 5000 unlinked markers and defined the probability of obtaining a false positive result as the proportion of the 5000 replicates for which we obtained a lod score higher than that observed for the original linked locus. All lod scores reported in this paper were obtained by converting the empirical *P* value obtained by simulation to its corresponding lod score ($\text{lod} = \chi^2/[2 \times \ln_{10}]$).

Power estimation

We performed a simulation study to evaluate the power of our sample to detect linkage. For this study we simulated genotypes for every pedigree member and phenotypes for those actually examined. The simulated genotypes consisted of a five-allele marker locus that was tightly linked to a two-allele obesity-causing QTL with a recombination fraction equal to zero. Allelic effects at the QTL were modeled to be additive. Eight different generating configurations with varying levels of heritability attributable to the QTL were simulated. For each generating configuration, we assumed the total heritability of the phenotype to be 40%, which approximates that estimated for BMI in our sample. We then simulated the heritability at the QTL for values ranging from 5–40%, in increments of 5%. For simulated QTL effects of less than 40%, a residual heritability was included so that the total heritability, ($h^2_{QTL} + h^2_{\text{residual}}$), was kept constant at 40%. The size of these gene effects is a function of the separation between the means of the two homozygous genotypes (in SD units) and the allelic frequencies at the QTL.

Two hundred replicates were simulated for each of the eight generating configurations. We analyzed each replicate dataset using variance component linkage analysis. The power to detect linkage was defined as the proportion of replicates for which we obtained a lod score of 2.0 or greater.

Results

The mean age of the 672 participating study subjects was 47.2 ± 15.7 yr and did not differ significantly between men and women (Table 1). Approximately 9% of men and 13% of women had diabetes (age-adjusted *P* = 0.03). Mean levels of BMI, waist circumference, percent fat, and leptin concentrations were significantly higher in women than in men (age and diabetes status-adjusted *P* < 0.001). The correlations (*r*)

TABLE 1. Clinical characteristics of study subjects by gender (mean \pm SD)

Characteristic (unit)	Men	Women
Number	298	374
Diabetes (%)	8.7	12.8 ^a
Age (yr)	47.5 \pm 15.5	46.9 \pm 15.9
BMI (kg/m ²)	26.4 \pm 3.7	27.9 \pm 5.4 ^b
Waist (cm)	94.6 \pm 10.5	89.7 \pm 11.5 ^b
% of fat mass	16 \pm 7	30 \pm 8 ^b
Ln (leptin level) (ng/mL)	1.05 \pm 0.72	2.55 \pm 0.73 ^b

^a Age-adjusted *P* < 0.05.

^b Age- and diabetes status-adjusted *P* < 0.001.

between these traits ranged from 0.30 (waist circumference and percent fat) to 0.83 (leptin and percent fat). Leptin concentrations were strongly correlated with BMI ($r = 0.66$); however, as shown in Fig. 1, there was considerable variation in leptin concentrations even among individuals with the same BMI. BMI-adjusted leptin levels were highly correlated with both percent fat ($r = 0.60$) and the original (*i.e.* unadjusted) leptin concentrations ($r = 0.76$), suggesting a potential link between BMI-adjusted leptin levels and obesity.

The heritabilities for BMI, waist circumference, percent fat, and leptin concentration were 0.41, 0.33, 0.36, and 0.40, respectively (all $P < 0.0001$), as we have previously reported (22). In addition, the heritability for BMI-adjusted leptin was 0.30 ($P < 0.0001$). These estimates indicate that a substantial portion of the variation in these traits can be attributed to the additive effects of genes and provide the rationale for conducting genome-wide linkage analysis. Table 2 and Fig. 2 show the maximum lod score for each trait and the chromosomal locations corresponding to these peaks. Peak lod scores were 1.68 ($P = 0.0027$) for BMI (chromosome 16p), 1.80 ($P = 0.0020$) for waist circumference (chromosome 14q), 1.61 ($P = 0.0032$) for percent fat (chromosome 3p), and 1.72 ($P = 0.0024$) for leptin concentrations (chromosome 16p). There were no prominent linkage signals detected for obesity when defined as a dichotomous trait (having a BMI ≥ 30 kg/m²; maximum lod = 1.05 on chromosome 8q). When we also adjusted for the effect of diabetes status in our linkage analysis, these results were virtually unchanged in terms of both magnitude of the lod score and location of the linkage peak.

To evaluate the genetic determinants of BMI-adjusted leptin concentrations, we also carried out a genome scan of this trait. In this analysis we included BMI as a covariate in the linkage model to control for its effect on leptin in addition to that of sex and age. As shown in Table 2, the strongest evidence for linkage was observed on chromosome 10 (71 cM) with a lod score of 2.73 ($P = 0.0002$). In addition, lod scores greater than 1.5 were observed in two other chromosomal regions: one on chromosome 7 (lod = 1.77 or $P = 0.0022$, at 164 cM) and the other on chromosome 14 (lod = 2.47 or $P = 0.0004$, at 86 cM). As before, the results were virtually unchanged after adjusting for the effect of diabetes

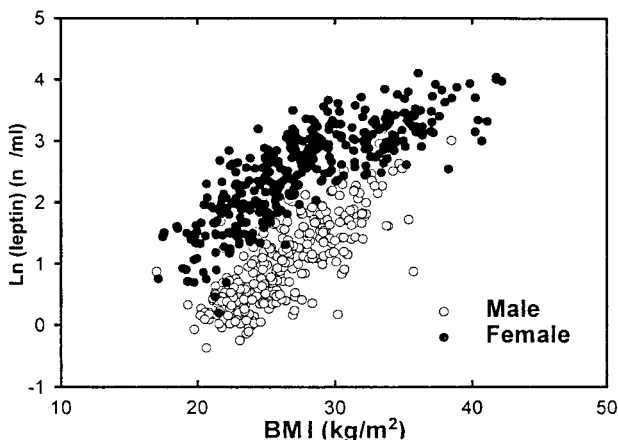


FIG. 1. The correlation between leptin concentrations and BMI ($r = 0.66$).

TABLE 2. Maximum lod scores from multipoint linkage analysis

Chromosome	Location (cM)	Trait	Lod score ^a
3	33	% Fat	1.61
7	164	BMI-adjusted leptin	1.77
10	71	BMI-adjusted leptin	2.73
14	52	Waist	1.80
	86	BMI-adjusted leptin	2.47
16	1	BMI	1.68
	14	Leptin	1.72

^a All lod scores are empirical lod scores based on simulations (see *Subjects and Methods*).

status. Results from the multipoint linkage analyses for 22 autosomes are shown in Fig. 2.

The results of our power simulation are summarized in Fig. 3. These results indicate that our sample provides good power (78%) to detect linkage (at lod scores >3.0) for a QTL accounting for 30% or more of the total trait variance. For a QTL accounting for 25% of the total trait variance, we would have 52% power to detect a lod score greater than 3.0 and 76% power to detect a lod score of 2.0 or greater. For a QTL accounting for 20% of the phenotypic variance, the power decreases dramatically (32% power to detect a lod score >3.0 and 52% to detect a lod score >2.0).

Discussion

Obesity is moderately heritable in the OOA, as it is in other populations (summarized in Ref. 22). Mean BMI levels in OOA males as well as in young OOA females (aged <40 yr) are, in general, comparable to those in the overall U.S. white population, although older OOA females (aged ≥ 40 yr) have mean BMI levels 1–2 kg/m² higher than those of U.S. white females in the same age group (1). Despite the well recognized genetic contribution to human obesity, the specific genes involved are unknown. We did not detect any lod scores at the significant level ($P < 0.05$ at a genome-wide context) for any of the traits we analyzed, perhaps reflecting the complexity of genetic mechanisms and/or the small effects of individual genes even in this relatively homogeneous population. However, the power of our family collection to detect QTL effects accounting for as high as 20–25% of total phenotypic variance was still quite low.

Peak lod scores for the primary obesity phenotypes that we analyzed (*i.e.* BMI, percent fat, waist circumference, and leptin) ranged between 1.6 and 1.8, occurring on chromosomes 3, 14, and 16. The inclusion of the diabetes status in our linkage model did not change the linkage results appreciably, suggesting the linkage signals we observed in this study may reflect genetic influences on obesity alone, rather than on both obesity and diabetes. Peak evidence for linkage to percent fat (lod = 1.61) occurred near marker *D3S3608* on chromosome 3p, less than 2 cM away from a linkage reported by Lee *et al.* (19) to this same trait ($P = 0.011$ estimated by single point analysis for marker *D3S1286*). This region is of particular interest because it corresponds to the location of the peroxisome proliferator-activated receptor- γ (*PPAR* γ) gene, which maps to chromosome 3p25–24.2, approximately 2 cM telomeric from the peak signal observed. The product

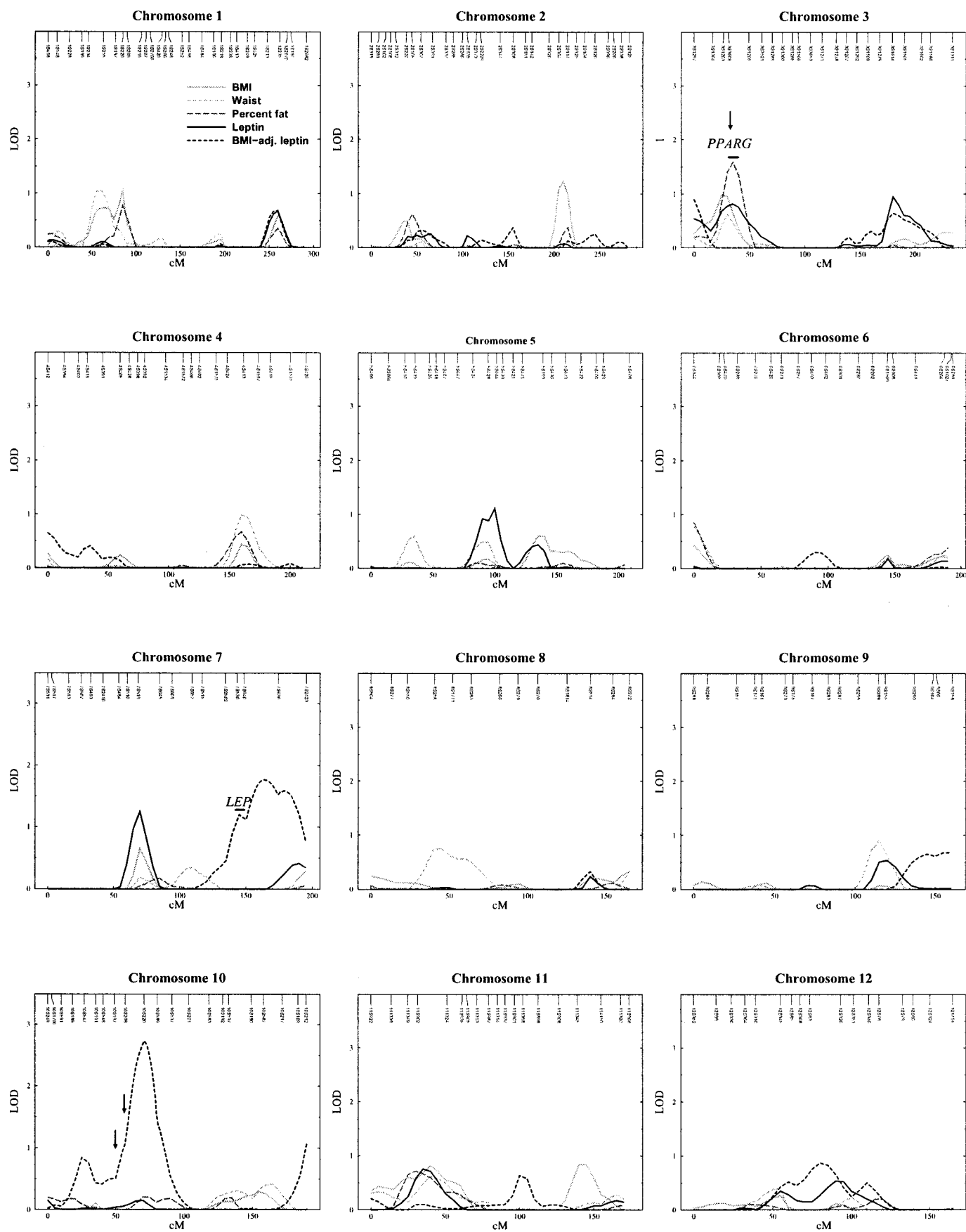


FIG. 2. Multipoint linkage analysis results for obesity-related traits for 22 autosomes. ↓, The locations of linkage signals reported in the literature. *PPARG* and *LEP* are the GenBank designations for the *PPAR γ* and leptin genes, respectively.

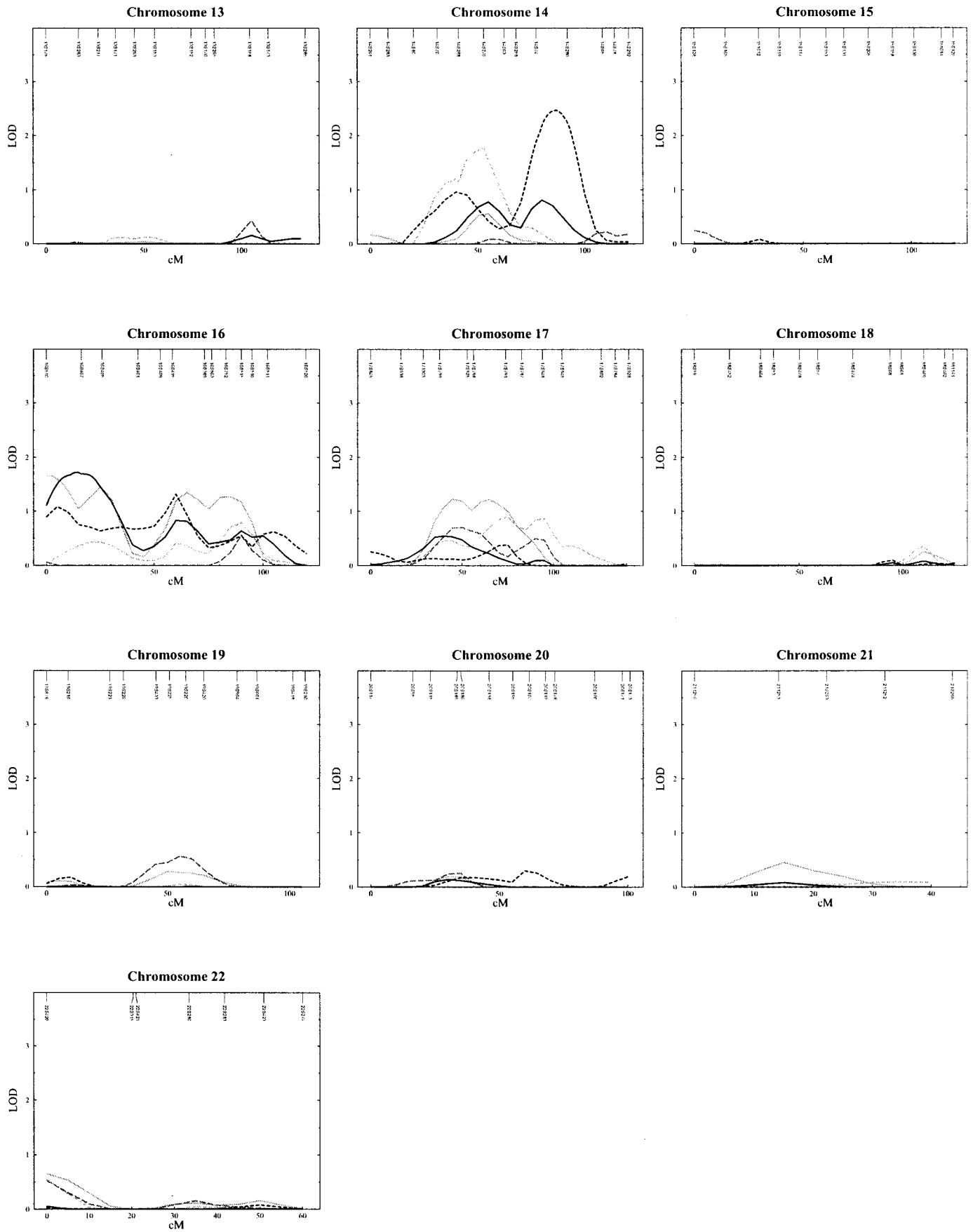


FIG. 2. Continued.

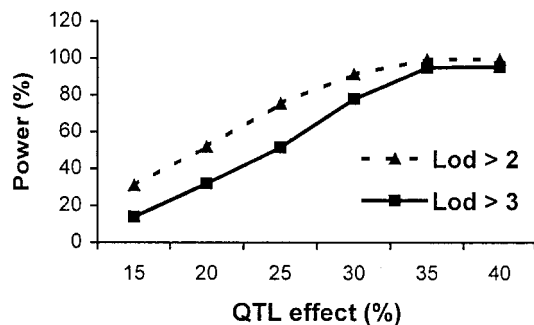


FIG. 3. Power to detect QTL effects in the OOA pedigrees.

of this gene is a nuclear hormone receptor that stimulates adipogenesis (27) and increases insulin sensitivity (28). To date, four variants causing amino acid changes and one silent mutation have been identified, and these variants have been associated in some populations with obesity and altered insulin sensitivity (29–33).

The highest lod scores we observed in our genome scan were for the trait, BMI-adjusted leptin levels. The peak lod score we observed was 2.73 ($P = 0.0002$), occurring on chromosome 10p. In addition, we observed lod scores of 2.47 and 1.77, occurring on chromosomes 14q and 7q, respectively. An obvious obesity candidate gene in the region of linkage on chromosome 7 is the leptin gene (*LEP*). *LEP* has been mapped to 7q31.3, which is within the broad range of the chromosome 7 linkage signal. Rare mutations in *LEP* have been shown to cause leptin deficiency and extreme obesity in humans (34, 35). A linkage study conducted in Mexican Americans also reported linkage of several obesity-related traits to the gene *LEP* region (36).

The relevance of BMI-adjusted leptin levels to obesity is controversial. Leptin is secreted by adipose tissue and appears to signal the central nervous system regarding available energy stores. The correlation between serum leptin concentration and BMI is high in the OOA ($r = 0.66$), as it is in other populations. This may reflect resistance to the action of leptin (37). Although an absolute deficiency of leptin is associated with extreme obesity in both mice (38) and humans (34, 35), there is also a subset of individuals who have a relative deficiency of leptin given their level of fatness (39, 40). There is some indication that a relative deficiency of leptin may be a risk factor for weight gain. Prospectively, low leptin levels predict weight gain in Pima Indians (41). Moreover, weight-reduced postobese subjects have lower leptin levels and lower rates of fat oxidation than weight-matched (but never obese) subjects, possibly explaining the propensity of weight-losers to regain weight they lost (42). The issue remains controversial, however, as at least two other prospective studies have reported that low leptin levels do not predict weight gain (43, 44).

The causes of relative deficiency (or relative excess) of leptin levels for a given BMI are largely unknown. To our knowledge, this is the first demonstration that BMI-adjusted leptin concentrations are significantly heritable, suggesting that there may be genetic influences on this trait. Presumably, a defect in leptin regulation could lead to secretory insufficiency, analogous to pancreatic β -cell defects leading to deficiencies in insulin secretion. Leptin regulation is complex.

Factors shown to influence the expression of leptin include intracellular glucosamine concentrations, tumor necrosis factor, insulin, glucocorticoids, interleukin-1m, c/EBP, and PPAR γ (45).

To date, genome-wide linkage studies of obesity-related traits have been reported in at least four other populations (13–19). A QTL influencing serum leptin concentrations on chromosome 2p21 was identified in Mexican American and French subjects (13, 18), a QTL on chromosome 8p for BMI in Mexican Americans (14), one locus on chromosome 10p for obesity in French subjects (18, 46), one QTL on chromosome 11q for BMI and percent body fat in Pima Indians (15, 16), and a QTL on chromosome 20q for BMI in white American (19). Interestingly, our peak signal for BMI-adjusted leptin concentrations is approximately 21 cM from a QTL influencing obesity (lod = 4.85) in a French population (18) and within 10–20 cM of an obesity locus reported in a German study (46). The linkages reported in the French and German studies were both telomeric to that observed for BMI-adjusted leptin levels in the Amish. In the German study the maximum multipoint lod score was 2.32, approximately 19 cM from the Amish peak, and the peak two-point lod score was 2.45 (to marker *TCF8*), approximately 9 cM away (46). It is difficult to evaluate whether a putative gene underlying the linkage signal in one study (e.g. the OOA) represents the same gene(s) as those detected by linkage in other studies (e.g. in the French and German studies). Simulation studies using an affected sibling pair design indicate that even when the genetic effect is strong (e.g. lod > 3 with a λ s of 2) and the sample size is large (400 affected sibling pairs, 10 cM map density), location estimates can be as far as 20 cM away from the true locus location (47, 48).

In summary, we obtained suggestive evidence for linkage to BMI-adjusted leptin concentrations on three chromosomal regions, yet little evidence for linkage was detected for other obesity-related traits. Of particular interest are regions on chromosomes 3, 7, and 10. These regions have already been shown to be linked to extreme obesity and/or obesity-related traits by one or more studies in other populations. The linkage signals on chromosomes 3 and 7 reside in regions where known candidate genes for obesity are located (*PPAR γ* and *LEP*, respectively). The linkage signals for the trait BMI-adjusted leptin concentrations may warrant further investigations for their potential relevance to the development of human obesity.

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References

1. Kuczmarski RJ, Flegal KM, Campbell SM, Johnson CL. 1994 Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys. 1960 to 1991. *JAMA*. 272:205–211.
2. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. 1999 The disease burden associated with overweight and obesity. *JAMA*. 282:1523–1529.
3. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW, Jr. 1999 Body-mass

- index and mortality in a prospective cohort of U.S. adults. *N Engl J Med.* 341:1097–1105.
4. **Wolf AM, Colditz GA.** 1998 Current estimates of the economic cost of obesity in the United States. *Obes Res.* 6:97–106.
 5. **Perusse L, Bouchard C.** 1999 Genotype-environment interaction in human obesity. *Nutr Rev.* 57:S31–S37.
 6. **Perusse L, Chagnon YC, Weisnagel J, Bouchard C.** 1999 The human obesity gene map: the 1998 update. *Obes Res.* 7:111–129.
 7. **Moll PP, Burns TL, Lauer RM.** 1991 The genetic and environmental sources of body mass index variability: the Muscatine Ponderosity Family Study. *Am J Hum Genet.* 49:1243–1255.
 8. **Rice T, Borecki IB, Bouchard C, Rao DC.** 1993 Segregation analysis of fat mass and other body composition measures derived from underwater weighing. *Am J Hum Genet.* 52:967–973.
 9. **Comuzzie AG, Blangero J, Mahaney MC, et al.** 1995 Major gene with sex-specific effects influences fat mass in Mexican Americans. *Genet Epidemiol.* 12:475–488.
 10. **Bouchard C, Perusse L, Leblanc C, Tremblay A, Theriault G.** 1988 Inheritance of the amount and distribution of human body fat. *Int J Obes.* 12:205–215.
 11. **Lecomte E, Herbeth B, Nicaud V, Rakotavoa R, Artur Y, Tiret L.** 1997 Segregation analysis of fat mass and fat-free mass with age- and sex-dependent effects: the Stanislas Family Study. *Genet Epidemiol.* 14:51–62.
 12. **Borecki IB, Higgins M, Schreiner PJ, et al.** 1998 Evidence for multiple determinants of the body mass index: the National Heart, Lung, and Blood Institute Family Heart Study. *Obes Res.* 6:107–114.
 13. **Comuzzie AG, Hixson JE, Almasy L, et al.** 1997 A major quantitative trait locus determining serum leptin levels and fat mass is located on human chromosome 2. *Nat Genet.* 15:273–276.
 14. **Mitchell BD, Cole SA, Comuzzie AG, et al.** 1999 A quantitative trait locus influencing BMI maps to the region of the β -3 adrenergic receptor. *Diabetes.* 48:1863–1867.
 15. **Norman RA, Tataranni PA, Pratley R, et al.** 1998 Autosomal genomic scan for loci linked to obesity and energy metabolism in Pima Indians. *Am J Hum Genet.* 62:659–668.
 16. **Hanson RL, Ehm MG, Pettitt DJ, et al.** 1998 An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet.* 63:1130–1138.
 17. **Walder K, Hanson RL, Kobes S, Knowler WC, Ravussin E.** 2000 An autosomal genomic scan for loci linked to plasma leptin concentration in Pima Indians. *Int J Obes.* 24:559–565.
 18. **Hager J, Dina C, Francke S, et al.** 1998 A genome-wide scan for human obesity genes reveals a major susceptibility locus on chromosome 10. *Nat Genet.* 20:304–308.
 19. **Lee JH, Reed DR, Li WD, et al.** 1999 Genome scan for human obesity and linkage to markers in 20q13. *Am J Hum Genet.* 64:196–209.
 20. **Cross HE.** 1976 Population studies and the Old Order Amish. *Nature.* 262:17–20.
 21. **Agarwala R, Biesecker LG, Hopkins KA, Francomano CA, Schaffer AA.** 1998 Software for constructing and verifying pedigrees within large genealogies and an application to the Old Order Amish of Lancaster County. *Genome Res.* 8:211–221.
 22. **Hsueh W-C, Mitchell BD, Aburomia R, et al.** 2000 Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care.* 23:595–601.
 23. **Wattanapenpaiboon N, Lukito W, Strauss BJ, Hsu-Hage BH, Wahlqvist ML, Stroud DB.** 1998 Agreement of skinfold measurement and bioelectrical impedance analysis (BIA) methods with dual energy x-ray absorptiometry (DEXA) in estimating total body fat in Anglo-Celtic Australians. *Int J Obes Relat Metab Disord.* 22:854–860.
 24. **Almasy L, Blangero J.** 1998 Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet.* 62:1198–1211.
 25. **Williams JT, Van Eerdewegh P, Almasy L, Blangero J.** 1999 Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. *Am J Hum Genet.* 65:1134–1147.
 26. **Hasstedt SJ.** 1993 Variance components/major locus likelihood approximation for quantitative, polychotomous, and multivariate data. *Genet Epidemiol.* 10:145–158.
 27. **Tontonoz P, Hu E, Spiegelman BM.** 1994 Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell.* 79:1147–1156.
 28. **Spiegelman BM.** 1997 Peroxisome proliferator-activated receptor γ : a key regulator of adipogenesis and systemic insulin sensitivity. *Eur J Med Res.* 2:457–464.
 29. **Beamer BA, Yen CJ, Andersen RE, et al.** 1998 Association of the Pro12Ala variant in the peroxisome proliferator-activated receptor- γ 2 gene with obesity in two Caucasian populations. *Diabetes.* 47:1806–1808.
 30. **Meirhaeghe A, Fajas L, Helbecque N, et al.** 1998 A genetic polymorphism of the peroxisome proliferator-activated receptor γ gene influences plasma leptin levels in obese humans. *Hum Mol Genet.* 7:435–440.
 31. **Ristow M, Muller-Wieland D, Pfeiffer A, Krone W, Kahn CR.** 1998 Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. *N Engl J Med.* 339:953–959.
 32. **Barroso I, Gurnell M, Crowley VEF, et al.** 1999 Dominant negative mutations in human PPAR γ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature.* 402:880–883.
 33. **Cole SA, Mitchell BD, Hsueh WC, et al.** 2000 The Pro12Ala variant of peroxisome proliferator-activated receptor- γ 2 (PPAR- γ 2) is associated with measures of obesity in Mexican Americans. *Int J Obes.* 24:522–524.
 34. **Montague CT, Farooqi IS, Whitehead JP, et al.** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature.* 387:903–908.
 35. **Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD.** 1998 A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet.* 18:213–215.
 36. **Duggirala R, Stern MP, Mitchell BD, et al.** 1996 Quantitative variation in obesity-related traits and insulin precursors linked to the OB gene region on human chromosome 7. *Am J Hum Genet.* 59:694–703.
 37. **Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV.** 1996 Leptin: the tale of an obesity gene. *Diabetes.* 45:1455–1462.
 38. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM.** 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature.* 372:425–432.
 39. **Maffei M, Halaas J, Ravussin E, et al.** 1995 Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med.* 1:1155–1161.
 40. **Considine RV, Sinha MK, Heiman ML, et al.** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 334:292–295.
 41. **Ravussin E, Pratley RE, Maffei M, et al.** 1997 Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med.* 3:238–240.
 42. **Filozof CM, Murua C, Sanchez MP, et al.** 2000 Low plasma leptin concentration and low rates of fat oxidation in weight-stable post-obese subjects. *Obes Res.* 8:205–210.
 43. **Haffner SM, Mykkanen LA, Gonzalez CC, Stern MP.** 1998 Leptin concentrations do not predict weight gain: the Mexico City Diabetes Study. *Int J Obes Relat Metab Disord.* 22:695–699.
 44. **Hodge AM, de Courten MP, Dowse GK, et al.** 1998 Do leptin levels predict weight gain? A 5-year follow-up study in Mauritius. Mauritius Non-communicable Disease Study Group *Obes Res.* 6:319–325.
 45. **Friedman JM, Halaas JL.** 1998 Leptin and the regulation of body weight in mammals. *Nature.* 395:763–770.
 46. **Hinney A, Ziegler A, Oeffner F, et al.** 2000 Independent confirmation of a major locus for obesity on chromosome 10. *J Clin Endocrinol Metab.* 85:2962–2965.
 47. **Hauser ER, Boehnke M.** 1997 Confirmation of linkage results in affected-sib-pair linkage analysis for complex trait. *Am J Hum Genet.* 61[Suppl 1]:A278.
 48. **Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS.** 1999 Replication of linkage studies of complex traits: an examination of variation in location estimates. *Am J Hum Genet.* 65:876–884.