

Eating behavior in the Old Order Amish: heritability analysis and a genome-wide linkage analysis¹⁻³

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ABSTRACT

Background: Eating behavior and thus dietary intake affect the development of obesity-related diseases such as diabetes, hypertension, and hyperlipidemia.

Objective: We investigated the genetic underpinnings of eating behavior.

Design: We administered a standardized eating behavior inventory to 624 adults from 28 families participating in the Amish Family Diabetes Study. Three quantifiable components of eating behavior were measured: restraint, disinhibition, and hunger. Associations between eating behavior scores and physical characteristics were evaluated. Heritability analysis and a genome-wide multipoint linkage analysis were performed.

Results: Eating behavior scores were associated with obesity and obesity-related phenotypes. Heritability estimates were 0.28 ± 0.09 for restraint, 0.40 ± 0.10 for disinhibition, and 0.23 ± 0.09 for hunger ($P < 0.001$). The linkage analysis showed 4 regions of suggestive linkage. We observed suggestive evidence for linkage of restraint scores to 2 chromosomal regions, near markers D3S1304 [LOD (log of odds) = 2.5, $P = 0.0003$] and D6S276 (LOD = 2.3, $P = 0.0006$). We previously reported that D3S1304 is linked to a locus influencing percentage body fat in this same population (LOD = 1.6), suggesting that this behavioral phenotype may be secondary to obesity. The maximum LOD scores for disinhibition were 1.6 ($P = 0.003$) near marker D7S657 and 1.4 ($P = 0.005$) near marker D16S752. The maximum LOD score for hunger was 1.4 ($P = 0.005$) near marker D3S1278.

Conclusion: Significant familial effects on eating behavior and suggestive genetic linkage were found in Amish adults. *Am J Clin Nutr* 2002;75:1098–1106.

KEY WORDS Restraint, obesity, behavioral genetics, Amish, heritability, linkage analysis

INTRODUCTION

In the United States, nutritional intake contributes to the development of human disease, mainly in the form of obesity-related conditions such as diabetes, hypertension, and hyperlipidemia. Although environmental, psychological, and physiologic factors affect appetite and nutrient intake, several studies suggest that behaviors such as food and beverage preferences and nutrient intake are at least partially genetically determined (1–4).

Reed et al (3) reviewed family and twin studies of food preferences and concluded that a genetic influence on food preference exists. These conclusions arise in part from twin studies showing that monozygotic twins have more similar protein and carbohydrate intakes than do dizygotic twins (4).

A few rare syndromes involving obesity and genetic mutations have been identified in humans (5). For example, Prader-Willi syndrome, an autosomal dominant disorder caused by a deletion in chromosome region 15q11.2–q12, is characterized by excessive eating and morbid obesity. Very rare monogenic forms of human obesity are due to mutations in the genes for leptin (6, 7), proconvertase 1 (8), leptin receptor (9), proopiomelanocortin (10), or melanocortin 4 receptor (11, 12), all of which result in a phenotype of excessive energy intake relative to expenditure. However, gene variants that increase the risk of typical obesity in humans remain largely unknown. Understanding the genetic basis for the control of eating behavior has implications for developing models of the pathophysiology of nutrition-related diseases and for prevention, diagnosis, and treatment of these disorders.

Eating behavior is difficult to define and quantify. The Three-Factor Eating Questionnaire (13, 14) measures 3 behavioral factors: restraint, disinhibition, and hunger. Restraint is a cognitive avoidance of eating to control body weight. An example of a true-or-false question that addresses restraint is “I do not eat some foods because they make me fat.” Disinhibition is loss of restraint resulting in overeating. An example of a disinhibition question is “When I am with someone who overeats I usually overeat too.” Hunger measures the perceived need for food, eg, “I get so hungry that my stomach often feels like a bottomless pit.”

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To examine the possible relations between eating behavior, obesity and related traits, and genetics, we administered the Three-Factor Eating Questionnaire to participants in the Amish Family Diabetes Study, a large family study investigating the genetics of type 2 diabetes and obesity. First, we evaluated the association between eating behavior and obesity phenotypes. Then we estimated the heritability of eating behavior. Finally, we conducted a genome-wide linkage analysis to identify chromosomal regions that are likely to harbor genes that regulate eating behavior traits.

SUBJECTS AND METHODS

Study population and subject recruitment

The Old Order Amish are mostly rural farmers and craftsmen. They lead a cultural and technologically distinct lifestyle. They are a genetically well-defined founder population with large families and well-documented genealogies (15). Family history records of the Amish in Lancaster County, PA, beginning from 1727 are highly preserved (16). Other features of this population include a relatively high standard of living, low migratory tendencies, and nonpractice of birth control, which facilitate the recruitment of large and extended families. These attributes make the Amish an ideal population in which to study genetic contributions to disease.

Subject recruitment for the Amish Family Diabetes Study began in early 1995. Details of the study design, recruitment, procedures, and pedigree construction were previously described (17). Subjects were enrolled through the Amish Research Clinic in Strasburg, PA. Individuals with type 2 diabetes were identified through door-to-door interviews and by word of mouth. Proband was defined as individuals with previously diagnosed diabetes with an age at diagnosis of 35–65 y. All first- and second-degree family members aged ≥ 18 y were recruited around the probands. If another individual with diabetes was identified in the family (eg, aunt or uncle), the family was expanded further to include the first- and second-degree relatives aged ≥ 18 y of that individual.

Because dietary education for management of diabetes may alter eating behavior scores, subjects with prevalent diabetes ($n = 30$) were excluded from these analyses. Thus, this report is based on data from 624 relatives of the probands who were not known to have diabetes at the time of their evaluation. Although all of our subjects can be related by tracing their ancestors back multiple generations (18), to reduce computational difficulties, we divided the sample into 28 discrete families, ranging in size from 3 to 69 individuals. The sample included a large number of relative pairs, including 436 parent-offspring pairs, 1326 sibling pairs, 1342 avuncular pairs (aunt or uncle with a niece or nephew), and 1311 first cousin pairs. The study was conducted in accordance with the standards involving human subjects of the Institutional Review Board of the University of Maryland, and informed consent was obtained from all subjects.

Phenotypic characterization

Data regarding medical and family history were obtained during one clinic visit. Medication history was reviewed. No subject was taking any medication known to affect appetite. Height and weight were measured with the use of a stadiometer and a calibrated scale. The subjects removed their shoes and wore light clothing for these measurements. Waist circumference was measured at the level of the umbilicus, and hip circumference was

measured at the widest protuberance across the pelvis. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) to estimate total adiposity. The waist-to-hip ratio (WHR) and subscapular-to-triceps skinfold thickness ratio were calculated as indexes of abdominal (central) adipose distribution. Percentage body fat was determined by bioimpedance with the use of standard formulas (17).

Fasting lipid profile (total cholesterol, HDL cholesterol, and triacylglycerols) was assayed by Quest diagnostics (Baltimore), and the interassay CVs were 1.6% for total cholesterol, 5.0% for HDL cholesterol, and 1.6% for triacylglycerols. Fasting leptin concentrations were assayed at the Johns Hopkins Bayview General Clinical Research Center core laboratory by radioimmunoassay (Linco, Inc, St Louis, MO; interassay CV: 4.25%). Oral glucose tolerance tests were administered to subjects with no history of diabetes.

The Three-Factor Eating Questionnaire was administered at the same visit. This questionnaire has 51 items, 36 true-false and the remainder asking for a rating on a scale of 1–4 or 1–5 (13, 14). Each question is assigned a weight of one point, and scores are categorized by eating behavior trait. The range of scores for restraint is 0–21, for disinhibition 0–16, and for hunger 0–14.

To our knowledge, the Three-Factor Eating Questionnaire has not been used previously to study the Amish. Therefore, we investigated the means and distributions of answers to each question as well as the overall scores for restraint, disinhibition, and hunger. We found that the responses to each question were distributed across the range of possible responses in the Amish. Furthermore, eating behavior scores spanned all possible values for each of the 3 behaviors, and the configuration of frequency of scores was compatible with a Gaussian distribution. Finally, we compared the mean scores from subjects in other studies with our results and found that the mean scores of Amish subjects were similar to those reported in other populations (14).

Analysis of polymorphic short tandem repeat markers

Whole blood was collected, and genomic DNA was isolated with the use of Qiagen Maxiprep and the manufacturer's instructions (Qiagen, Inc, Santa Clarita, CA). For our genome scan we used fluorescent semiautomated genotype analysis with the Prism Linkage Mapping Set (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). Polymerase chain reactions were performed on 25 ng genomic DNA with the use of 96-well plates. Polymerase chain reaction mixtures were pooled according to the manufacturer's recommendations and loaded onto a 5% denaturing polyacrylamide gel (Long Ranger; FMC Bioproducts, Rockland, ME) positioned within the ABI 377 automated DNA sequencer (Applied Biosystems Division, Perkin-Elmer). Polymerase chain reaction failure rates were low (generally $< 1\%$). The gel runs were visually inspected and were analyzed with the use of ABI GENESCAN software (Applied Biosystems). The inspected data were imported into ABI GENOTYPER software (Applied Biosystems), and alleles were assigned and binned. To assess genotyping accuracy, several pairs of replicate DNA samples were genotyped. On the basis of blind replicates of the 373 markers (357 autosomal and 16 X chromosome) that were used in the linkage analysis, the genotyping error rate was 0.16%. Mendel error checks were performed with the use of MENDEL (19); we detected no cases of nonpaternity. The small

TABLE 1
Clinical characteristics of Amish subjects by sex¹

Characteristic	Men (n = 286)	Women (n = 338)
Age (y)	46 ± 15	45 ± 15
BMI (kg/m ²)	26.4 ± 3.7	28.1 ± 5.4 ²
Waist circumference (cm)	95 ± 10	89 ± 5 ²
Waist-to-hip ratio	0.90 ± 0.05	0.82 ± 0.06 ²
Body fat (%)	15.0 ± 8.0	29.1 ± 11.2 ²
Leptin (µg/L)	3.7 ± 3.2	16.2 ± 11.1 ²
Blood pressure (mm Hg)		
Systolic	121 ± 15	122 ± 18
Diastolic	79 ± 10	79 ± 9
Total serum cholesterol (mmol/L)	5.53 ± 1.24	5.48 ± 1.16
HDL cholesterol (mmol/L)	1.21 ± 0.34	1.40 ± 0.34 ²
Triacylglycerols (mmol/L)	0.90 ± 0.61	0.93 ± 0.54
Fasting insulin (pmol/L)	10 ± 4	11 ± 7

¹ $\bar{x} \pm$ SD. All traits except age and BMI were adjusted for age and BMI.

²Significantly different from men, $P < 0.001$ (ANOVA).

number of genotypes exhibiting Mendel errors that could not be resolved by visual inspection of the gel were archived and deleted from the database before linkage analysis. On average, we obtained usable genotypes from $96.3 \pm 2.4\%$ ($\bar{x} \pm$ SD; range: 81.9–99.8%) for each of the 373 markers used in the linkage analysis. The marker order and distances between markers were estimated from our data with the use of CRIMAP (20). The average interval between markers was 10 centimorgans (cM), and the largest gap between markers was 25.4 cM, occurring on chromosome 7. The mean marker heterozygosity was 0.75 (range: 0.33–0.91).

Statistical methods

Association of eating questionnaire scores with clinical characteristics

The clinical characteristics of the study subjects were analyzed according to sex. The statistical significance of differences between men and women was tested with the use of the analysis of variance procedure in SYSTAT 8.0 (SPSS Inc, Chicago), adjusting for the effects of age and BMI. Two-factor analysis of variance was used to determine whether eating behavior scores were associated with sex or BMI.

We next conducted more complex analyses that took into account the complicated pedigree structure to estimate the effect of each eating behavior score on each measure of obesity. A series of models were considered, each including a single obesity measure as the dependent variable and a single eating behavior score as the independent variable. We then used maximum likelihood methods to estimate the association between eating behavior score and obesity measure while simultaneously adjusting for the effects of age and sex. Analyses were conditioned on the pedigree structures to account for the nonindependence between related individuals. We tested the statistical significance of the association by likelihood ratio test; that is, we compared the likelihood of the pedigree data under a full model (allowing the effect of eating behavior on obesity to be estimated) with that under a nested model (constraining the effect of eating behavior to be zero). Two times the difference between the log-likelihoods of the 2 models is distributed asymptotically as a chi-square distribution with degrees of freedom equal to the difference in the number of dependent factors in the models being compared. In our analysis,

the chi-square tests had one degree of freedom. These tests were performed with the use of the SOLAR program (21).

Heritability and linkage analyses

Heritabilities of the eating behavior scores were assessed as the proportion of total phenotypic variance that could be accounted for by the covariance among relatives, while simultaneously adjusting for the effects of age, sex-specific age, and age². We used maximum likelihood procedures as implemented in the SOLAR software package (21) for these analyses. We also considered the possibility that the familial effects on eating behavior may be due to shared household rather than shared genes. We therefore estimated the household effects on eating behavior by assigning a unique household number to individuals sharing the same street address at the time of their examination. The household effect was parameterized as a random effect by assigning a 1 if the relative pair shared the same household and a 0 if they did not.

We carried out quantitative trait locus (QTL) linkage analysis with the use of a variance components method in which variation in eating behavior scores was partitioned into components attributable to environmental covariates, the additive effects of genes (ie, residual heritability), a specific QTL (ie, the linkage component), and random errors. For these analyses, also, we used maximum likelihood procedures as implemented in the SOLAR software package (21). The additive genetic effect 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 relations at the marker locus. The hypothesis of linkage was evaluated by the likelihood ratio test, in which we evaluated whether the locus-specific effect was significantly greater than zero (ie, $H_0: \sigma^2_{QTL} = 0$ compared with $H_A: \sigma^2_{QTL} > 0$). Both multipoint and two-point linkage analyses were carried out. Scores for restraint, disinhibition, and hunger were analyzed separately, and in each analysis, we simultaneously adjusted for the effects of age, sex-specific age, and age² on the outcome score. Because nonnormality of the data distribution can artificially inflate nominal LOD (log of odds) scores, we derived the distribution of LOD scores under the null hypothesis of no linkage by Monte Carlo simulation (22). Briefly, we simulated an unlinked marker locus with 5 equifrequent alleles, assigned genotypes to each founder, and then dropped genotypes down through the pedigree on the basis of Mendelian expectations and the founder genotypes. We then conducted linkage analysis of each eating behavior score with the simulated genotypes. We conducted 5000 replicates and defined the probability of obtaining a false positive result as the proportion of replicates for which we obtained a specified LOD score or higher. These probabilities were converted back into LOD scores, and these LOD scores can be interpreted as the probabilities of detecting linkage to an unlinked marker.

RESULTS

The clinical characteristics of the study population by sex are shown in **Table 1**, and the mean scores of the Three-Factor Eating Questionnaire are shown according to sex and BMI stratification in **Table 2**. Within each stratum of BMI, restraint scores were higher in women than in men. Restraint and disinhibition scores were significantly associated with both sex ($P < 0.001$) and BMI ($P < 0.001$), and hunger scores were associated with BMI ($P < 0.001$) but not with sex. There was no evidence for a sex-by-BMI interaction on any of the eating behavior scores.

TABLE 2
Three-Factor Eating Questionnaire scores of Amish subjects by BMI and sex¹

Eating behavior factor	BMI < 25		25 ≤ BMI < 30		BMI ≥ 30	
	Men (n = 117)	Women (n = 105)	Men (n = 116)	Women (n = 123)	Men (n = 53)	Women (n = 108)
Restraint	4.3	7.4	5.3	7.7	7.0	9.9
Disinhibition	3.6	4.6	4.5	5.4	6.3	7.3
Hunger	4.4	4.5	4.0	4.3	5.9	4.9

¹BMI data were missing for 2 subjects; therefore, mean eating behavior scores are reported for 622 subjects. Sex was significantly associated with restraint and disinhibition scores ($P < 0.001$), and BMI was significantly associated with restraint, disinhibition, and hunger scores ($P < 0.001$) by two-way ANOVA. There were no significant sex-by-BMI interactions.

The association between obesity and eating behavior scores for 5 different measures of obesity is shown in **Table 3**. Eating behavior scores were significantly and positively associated with each obesity trait, with one exception: restraint scores were not associated with the WHR. The association between the WHR and scores of disinhibition and the association between percentage body fat and scores of hunger were of only borderline significance.

The heritabilities of physical and physiologic characteristics as well as eating behavior scores are shown in **Table 4**. The heritabilities for restraint, disinhibition, and hunger were 0.28, 0.40, and 0.23, respectively. By comparison, the heritabilities of BMI, plasma leptin, and waist circumference were roughly 0.40, and those of plasma insulin and WHR were as low as 0.10–0.15. Inclusion of a household effect did not significantly reduce the variation in either restraint or hunger score, regardless of whether heritability was jointly estimated in the model. In contrast, there was a modest but significant effect of household on the disinhibition score if heritability was not included in the model ($P = 0.02$). However, adding a household effect to a model that already included heritability did not significantly improve the fit of the model to the data.

TABLE 3
β-Coefficients reflecting the association between obesity phenotypes and Three-Factor Eating Questionnaire scores in Amish subjects¹

Phenotype and eating behavior factor ²	Effect per score	P
BMI		
Restraint	0.29 ± 0.05	<0.0001
Disinhibition	0.59 ± 0.07	<0.0001
Hunger	0.31 ± 0.07	<0.0001
Waist		
Restraint	0.52 ± 0.12	<0.0001
Disinhibition	1.22 ± 0.16	<0.0001
Hunger	0.79 ± 0.17	<0.0001
Waist-to-hip ratio		
Restraint	0.001 ± 0.001	NS
Disinhibition	0.002 ± 0.001	0.03
Hunger	0.002 ± 0.001	0.008
Body fat (%)		
Restraint	0.33 ± 0.11	0.004
Disinhibition	0.47 ± 0.16	0.003
Hunger	0.33 ± 0.17	0.05
ln Leptin		
Restraint	0.04 ± 0.01	<0.0001
Disinhibition	0.07 ± 0.01	<0.0001
Hunger	0.04 ± 0.01	0.007

¹± SE; n = 624. Adjusted for the effects of age, sex-specific age, and age².

²Range of scores for restraint, 0–21; for disinhibition, 0–16; for hunger, 0–14.

Because eating behavior traits were significantly heritable, we hypothesized that variants at specific loci might be associated with these behaviors. To identify QTLs for eating behavior traits, we performed a genome-wide linkage analysis by using polymorphic short tandem repeat markers. Through these analyses we identified loci on 4 chromosomes as possible regions containing genes for eating behaviors. The results of the multipoint linkage analysis are summarized in **Table 5** and **Figures 1** and **2**. Additional details of these results may be obtained from the University of Maryland School of Medicine website (23).

The maximum LOD score for restraint was 2.5, occurring on chromosome 3 ≈ 19 cM from the p terminus near marker D3S1304. The 2-point LOD scores for markers in this region were 2.29 (D3S1304 at position 16.7 cM), 1.04 (D3S1263 at position 26.7 cM), and 2.64 (D3S3608 at position 31.0 cM). We also detected suggestive evidence for linkage of restraint scores to a region on chromosome 6, ≈ 40 cM from the p terminus near marker D6S276 (LOD = 2.3). The 2-point LOD scores for markers in this region were 0.91 (D6S470 at position 22.6 cM), 1.12 (D6S289 at position 32.9 cM), and 1.98 (D6S276 at position 46.5 cM). Evidence for linkage to the other eating behavior traits was less impressive. For disinhibition scores, the maximum multipoint LOD score occurred on chromosome 7 near marker D7S657 (LOD = 1.6) and on chromosome 16 near marker D16S752 (LOD = 1.4). For hunger scores, the maximum multipoint LOD score was 1.4, occurring on chromosome 3 near marker D3S1278.

DISCUSSION

The Three-Factor Eating Questionnaire has been used to predict success in weight-loss programs and to assess relations between

TABLE 4
Heritabilities (h^2) of eating behavior and other relevant traits of Amish subjects¹

Phenotype or eating behavior factor	h^2	P
Restraint	0.28 ± 0.09	<0.001
Disinhibition	0.40 ± 0.10	<0.001
Hunger	0.23 ± 0.09	<0.001
BMI	0.42 ± 0.07	<0.0001
Leptin	0.42 ± 0.07	<0.0001
Waist circumference	0.37 ± 0.07	<0.0001
Waist-to-hip ratio	0.13 ± 0.05	<0.0005
Diastolic blood pressure	0.24 ± 0.07	<0.0001
Total cholesterol	0.54 ± 0.08	<0.0001
ln Triacylglycerol	0.35 ± 0.07	<0.0001
ln Fasting insulin	0.11 ± 0.06	<0.05

¹± SE, n = 624. Adjusted for age, sex-specific age, and age².

TABLE 5Summary of all maximum log of odds (LOD) scores for eating behaviors, ≥ 1.4 , from multipoint genome-wide linkage analysis in Amish subjects¹

Eating behavior factor	Multipoint linkage analysis			
	LOD max (<i>P</i>)	Chromosome	Location <i>cM</i>	Closest marker
Restraint	2.5 (0.0003)	3	19	D3S1304
	2.3 (0.0006)	6	40	D6S276
Disinhibition	1.6 (0.0033)	7	105	D7S657
	1.4 (0.0052)	16	86	D16S752
Hunger	1.4 (0.0053)	3	120	D3S1278

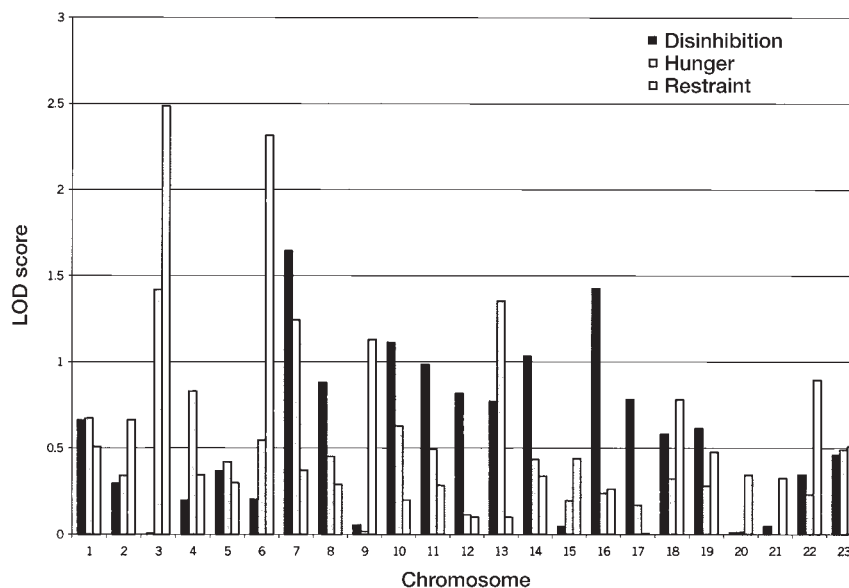
¹*n* = 624. cM, centimorgan.

eating behavior and daily energy intake, binge eating and other eating disorders, changes in weight, depression, smoking cessation, and cancer treatment (14, 24–28). This questionnaire has been validated in other populations but not among the Old Order Amish. However, on the basis of comparisons with other studies in which this tool was used (13, 14, 24–28), we believe that the Three-Factor Eating Questionnaire is relevant to the Amish and measures the intended behavioral phenotypes relevant to obesity and obesity-related traits. No published data exist on the frequency of eating behavior disorders in the Old Order Amish. However, our observations suggest the prevalence to be very low.

As in a Swedish study by Bjorvell et al (28), disinhibition scores in the present study were higher in obese persons than in normal-weight subjects. Because the brain influences food intake, it is probable that certain inherent behavioral traits, such as a tendency for disinhibition, may promote obesity in the appropriate environment. However, some studies showed changes in the scores for restraint, disinhibition, and hunger in response to treatment for obesity (14, 24), suggesting that eating behavior is pliable to some extent. Some studies also found a negative correlation between restraint and mean daily energy intake (14, 24), suggesting that restraint may serve to counteract weight gain. However, personality traits that favor dietary restraint may unintentionally promote weight gain by precipitating disinhibition and overeating (14). In the present study, we

found that obesity was associated with high restraint scores. Design features may have improved our ability to detect a relation that has eluded other investigators. It is likely that the influence of body composition on eating behavior is culturally determined and that a reduced stigmatization of obese individuals among the Old Order Amish may diminish the influence of obesity on behavior and thus make the influence of inherent behavioral traits on body composition more apparent. A large number of subjects is another advantage of the present study. To avoid the confounding effect of professional dietary advice, subjects with prevalent diabetes (*n* = 30) were excluded. In contrast with other studies, ours did not select subjects on the basis of their interest in a weight-loss program.

The evidence for a genetic component to eating behaviors comes from our heritability and linkage analyses. Heritability was highest for disinhibition, with the magnitude of the additive genetic effects rivaling that of many nonbehavioral measures of obesity (29). Disinhibition was also the factor that showed the strongest association with obesity phenotypes. To the extent that related persons may also live together, it could be argued that a component of the heritability between related individuals is attributable to their shared household. With the exception of disinhibition, we found that the contribution of household effect to our heritability estimates of eating behavior traits was negligible, supporting the theory that biological rather than social inheritance

**FIGURE 1.** Maximum log of odds (LOD) scores by chromosome and eating behavior trait from multipoint linkage analysis in 624 Amish subjects.

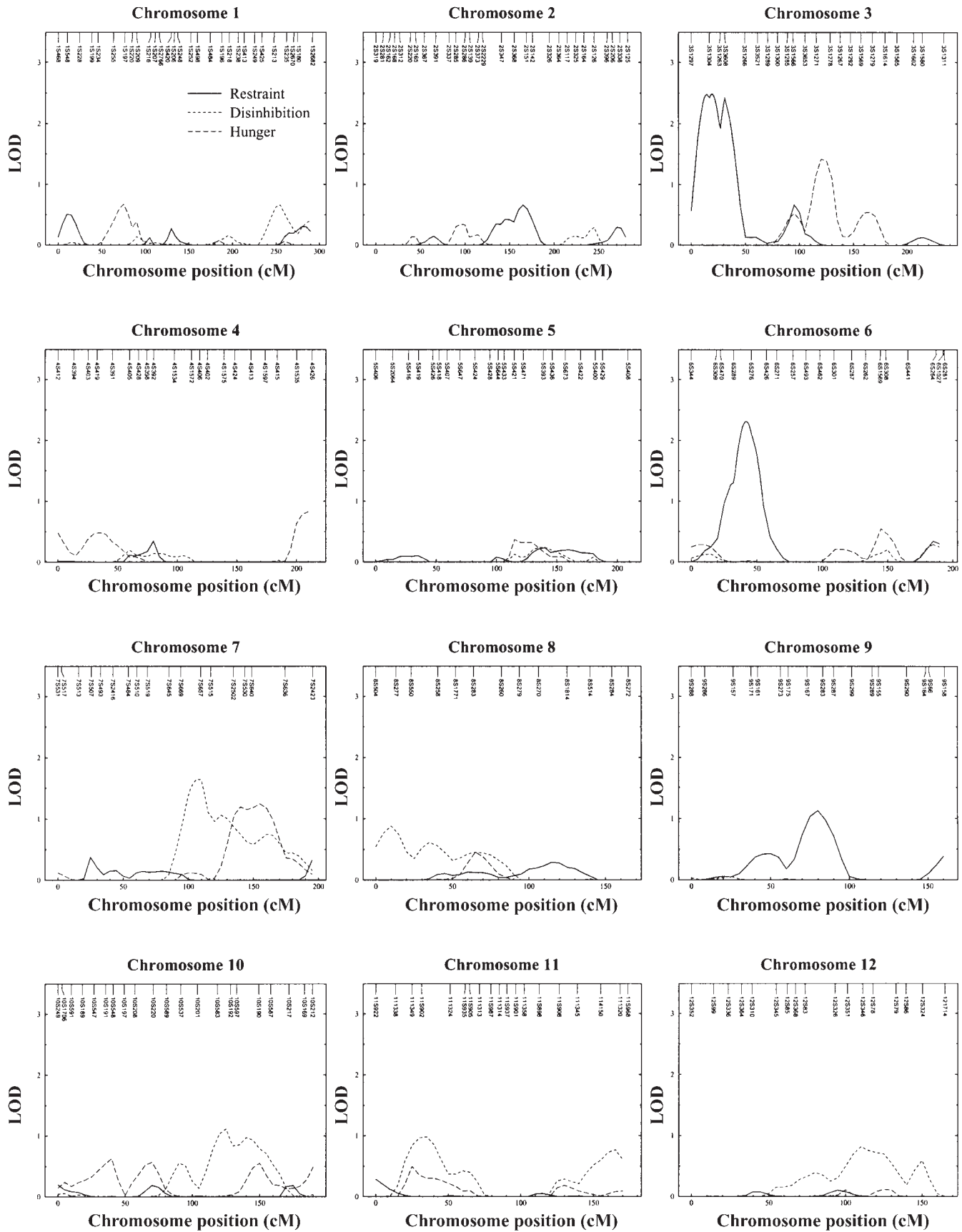


FIGURE 2. Variance components linkage analysis of eating behavior traits in 624 Amish subjects. cM, centimorgan; LOD, log of odds. (Continued)

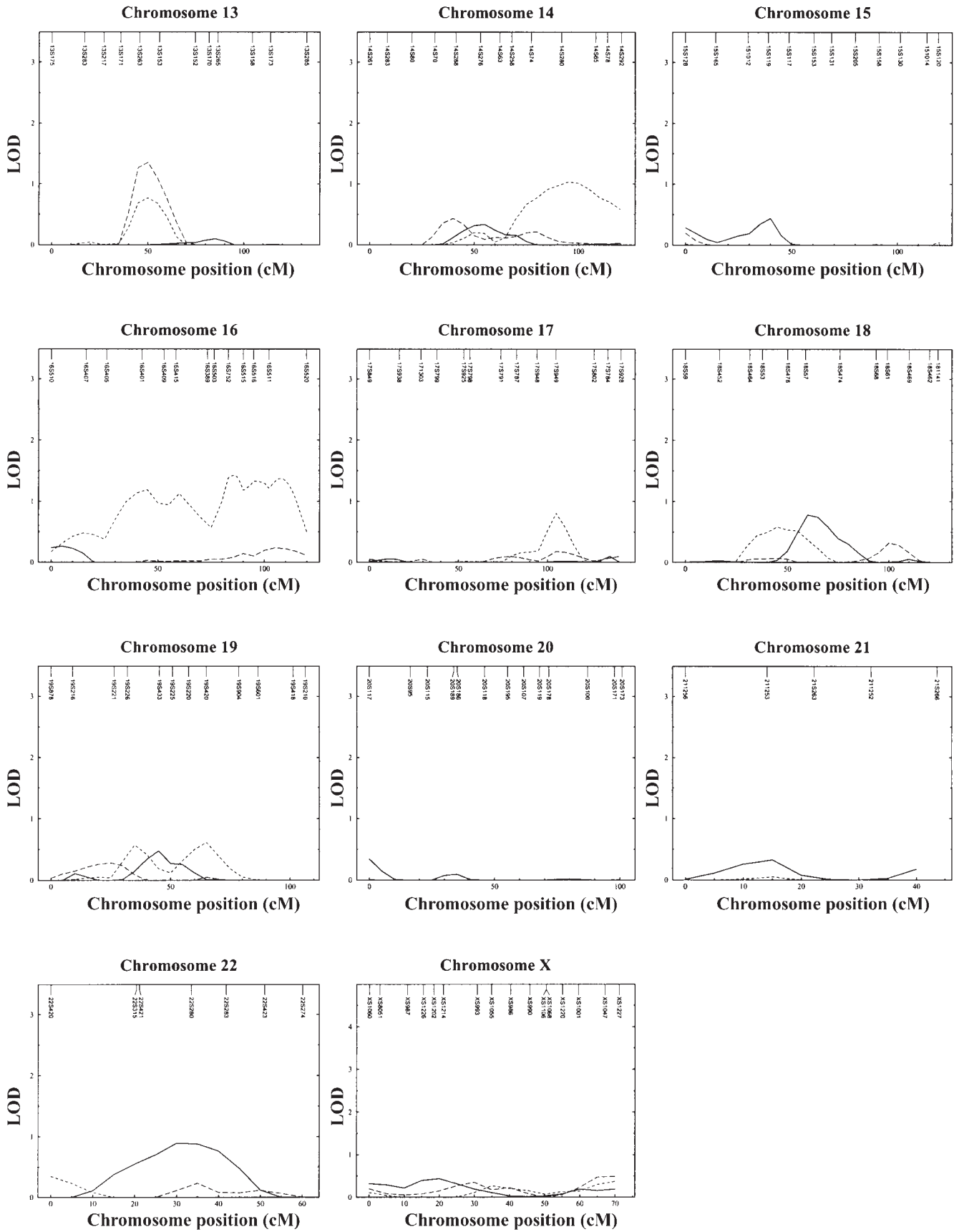



FIGURE 2.

explains the observations. Although subjects with diabetes were specifically excluded because of potential effects of nutrition counseling on eating behaviors, it is possible that households with diabetic subjects may exhibit a familial disinhibition that results from a shared effect of the nutrition counseling provided to the diabetic family member.

Linkage of eating behavior traits to specific chromosomal regions further suggests that specific genetic variants may account for some of these genetic effects. However, these results must be interpreted cautiously. Evidence for linkage was expressed in terms of LOD scores, defined as the logarithm of the ratio of the likelihood for linkage to the likelihood for no linkage. Some experts have proposed that when many genetic markers are analyzed simultaneously (as in genome-wide searches), LOD-score thresholds of 3.3 and 1.9 be used to assert evidence for significant linkage and suggestive linkage, respectively (30). It is becoming increasingly clear that for complex traits, these criteria are likely to be too rigorous. Importantly, the LOD scores that we report were derived by simulation studies; thus, they correspond to specific empirically derived *P* values that are not likely to be inflated. The relevance of these linkages must await replication by others or identification of the actual gene variants accounting for phenotypic variation in eating behavior traits.

The burgeoning Human Genome Project database has provided the opportunity to identify many of the genes that reside within the putative regions of linkage. Indeed, several candidate genes in the regions of these linkages may influence eating behavior. However, because we do not yet know all of the genes expressed within these regions, nor do we know the functions of many of the genes that reside in these regions, examination of positional candidate genes must be regarded with caution. A functional candidate gene on chromosome 3, which is located within our region of peak linkage to disinhibition at 10.38–10.5 cM, encodes peroxisome proliferator activated receptor γ (PPAR- γ). PPAR- γ is expressed in many tissues, but the highest concentrations are found in adipose tissue and large intestine (31). PPAR- γ is an important mediator of adipocyte differentiation and energy storage. PPAR- γ agonists stimulate eating by decreasing leptin concentrations (32). Furthermore, it was shown that mutations in PPAR- γ are associated with obesity (33, 34). Indeed, we found modest evidence for linkage of this same region on chromosome 3 to obesity-related traits in the Amish (29). We also found a linkage for restraint on chromosome 6. The area of linkage lies in proximity to the genes encoding glucagon-like peptide 1 (GLP-1) receptor, tumor necrosis factor α (TNF- α), and lymphotoxin. GLP-1 was shown to be involved in eating behavior in human and animal models (35, 36). GLP-1 receptors are found in the hypothalamus and are the target for hypothalamic neurons containing glucose transporter 2 and glucokinase. GLP-1 secretion after a meal stimulates insulin release, lowers blood glucose, and reduces food intake (36). TNF- α and lymphotoxin are cytokines known to be expressed in adipocytes. TNF- α may have a role in the development of insulin resistance and obesity (37–39). Evidence of association and linkage of the TNF- α gene with percentage body fat and BMI was reported by others (5). Linkage analysis of disinhibition scores yielded suggestive evidence for linkage on chromosome 7. Interestingly, the region of linkage lies in proximity to the leptin and plasminogen activator inhibitor 1 (PAI-1) genes. Other researchers found evidence of association of leptin with weight loss, body weight, leptin concentrations, and leptin concentration in response to diet

(5, 39–43). PAI-1 plays a role in the development of coronary artery disease. Plasma concentrations of PAI-1 may be higher in persons with obesity, insulin resistance, and type 2 diabetes and in their family members (45, 46).

To our knowledge this is the largest family study of eating behavior published to date. We found that eating behavior is familial in the Amish and for the first time show linkage of specific chromosomal regions to eating behavior traits. Replication of these studies in other populations and ultimately the positional cloning of the genes involved will be required to confirm and extend our findings. Insights gained by identifying genes that influence eating behavior may provide new molecular targets for the early detection of individuals susceptible to obesity, which may advance the development of preventive interventions and provide new drug targets for the treatment of obesity and related disorders. 

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