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Autosomal Genomic Scan for Loci Linked to Obesity and Energy Metabolism in Pima Indians

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Summary

An autosomal genomic scan to search for linkage to obesity and energy metabolism was completed in Pima Indians, a population prone to obesity. Obesity was assessed by percent body fat (by hydrodensitometry) and fat distribution (the ratio of waist circumference to thigh circumference). Energy metabolism was measured in a respiratory chamber as 24-h metabolic rate, sleeping metabolic rate, and 24-h respiratory quotient (24RQ), an indicator of the ratio of carbohydrate oxidation to fat oxidation. Five hundred sixteen microsatellite markers with a median spacing of 6.4 cM were analyzed, in 362 siblings who had measurements of body composition and in 220 siblings who had measurements of energy metabolism. These comprised 451 sib pairs in 127 nuclear families, for linkage analysis to obesity, and 236 sib pairs in 82 nuclear families, for linkage analysis to energy metabolism. Pointwise and multipoint methods for regression of sib-pair differences in identity by descent, as well as a sibling-based variance-components method, were used to detect linkage. LOD scores $\geqslant 2$ were found at 11q21-q22, for percent body fat (LOD = 2.1; $P = .001$), at 11q23-q24, for 24-h energy expenditure (LOD = 2.0; $P = .001$), and at 1p31-p21 (LOD = 2.0) and 20q11.2 (LOD = 3.0; $P = .0001$), for 24RQ, by pointwise and multipoint analyses. With the variance-components method, the highest LOD score (LOD = 2.3 $P = .0006$) was found at 18q21, for percent body fat, and at 1p31-p21 (LOD = 2.8; $P = .0003$), for 24RQ. Possible candidate genes include *LEPR* (leptin receptor), at 1p31, and *ASIP* (agouti-signaling protein), at 20q11.2.

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Introduction

Genetic linkage studies are focusing increasingly on complex traits, in the hope of uncovering one or more major contributing genes. Obesity is such a trait and is usually defined in terms of body-mass index (BMI), as excess weight for height (measured as kg/m^2), or, more accurately, in terms of body composition as excess adipose tissue. Obesity is increasing in Western societies and is responsible for a great deal of morbidity and mortality (Pi-Sunyer 1991). The strongest evidence for a genetic component to obesity comes from twin studies in which the heritability (H^2) has been estimated. Four studies of MZ twins reared apart (Stunkard et al. 1990; Allison et al. 1996) had led to the conclusion that BMI is heritable, with $H^2 = .50–.70$ (Allison et al. 1996). Although easily measured, BMI does not distinguish between fat and skeletal muscle and may not accurately indicate differences in body composition (Garn et al. 1986). At present, H^2 estimates for body composition are fewer in number. In the Quebec Family Studies, the estimate of H^2 for percent body fat was .25, whereas the estimate of H^2 for BMI was .05 (Bouchard et al. 1988). Because they were based on data from extended relatives, these values may be spuriously low (Allison et al. 1996). Familiality, defined as additive genetic effects plus shared environmental effects, of percent body fat estimated on the basis of relative similarities in the Pima Indians was .76, suggesting a genetic component in this population (Sakul et al. 1997).

Idiopathic obesity—that is, obesity having unknown cause—is the result of multiple behavioral and metabolic factors, including energy intake, energy expenditure, and the relative proportions of lipid and carbohydrate oxidized. Studies in Pima Indians have shown that low resting metabolic rate (Ravussin et al. 1988), low spontaneous physical activity (Zurlo et al. 1992), and a low ratio of fat oxidation to carbohydrate oxidation (Zurlo et al. 1990) are all predictors of weight gain (Ravussin and Swinburn 1993). Resting and 24-h metabolic rates

Table 1

Traits and Sample Sizes

Trait or Typing	No. of		
	Families	Siblings	Sib Pairs
Marker genotypes	127	482	966
%FAT[a]	127	362	451
W/T[a]	121	342	419
24EE[a]	82	220	236
SMR[a]	80	214	227
24RQ[a]	82	220	236

[a] After adjustment for appropriate covariates (see Subjects and Methods section).

show a familial aggregation (Bogardus et al. 1986; Rice et al. 1996*a*), and twin studies of resting metabolic rate have found concordance rates higher among MZ than among DZ twins (Fontaine et al. 1985; Bouchard et al. 1989). In addition, the respiratory quotient, the ratio of carbon dioxide production to oxygen consumption, is an indicator of the ratio of carbohydrate oxidation to fat oxidation, or nutrient partitioning, and is familial in Pima Indians. (Zurlo et al. 1990). Although the complex nature of obesity may impede attempts to find genes that contribute to it, metabolic traits that contribute to obesity may themselves be determined by smaller sets of genes and may constitute more-proximal manifestation of obesity genes. Thus, searches for genetic linkages with traits such as metabolic rate and respiratory quotient may facilitate identification of genetic variation underlying obesity.

Although there is little empirical evidence for genes with major effects, theoretical approaches using complex segregation analyses have indicated that major genes may affect fat mass and resting metabolic rate in French Canadians (Rice et al. 1993, 1996*b*), fat mass in Mexican Americans (Comuzzie et al. 1995), and BMI in Pima Indians (Price et al. 1994). Compared with that in other groups of North Americans, the BMI of Pima Indians is higher for most age groups (Knowler et al. 1991). However, the variation in BMI is comparable to that found within other North American groups (Kuczmarski et al. 1994), with an average SD of 7.1 (vs. 6.8) kg/m^2 in Pima males and 8.1 (vs. 8.7) kg/m^2 in Pima females. This indicates that, despite the high prevalence of obesity, Pima Indians represent a suitable population for study of the genetics of obesity. Preliminary results of an autosomal genomic scan for linkage to obesity in Pima Indians has been presented elsewhere (Norman et al. 1997). That study presented linkage analysis of individual markers for a single trait, percent body fat adjusted for sex and age (%FAT), in 88 nuclear families, with follow-up multipoint analysis on selected regions. In the present report, we present the results of an extended genomic scan, including an additional 39 nuclear families, for linkage to %FAT, fat distribution, and energy metabolism. We also fully analyze the data, using two multipoint methods—one based on sib-pair differences and the other based on sibling variance components—using a microsatellite map derived from genetic data on Pima Indians.

Subjects and Methods

Linkage Studies

Subjects were selected from ongoing longitudinal studies of type 2 diabetes in the Pima Indians (Knowler et al. 1990). All studies were approved by the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Community. The analysis of obesity and energy metabolism includes the 127 families (362 nondiabetic siblings) with at least two offspring, measured for %FAT (table 1). Of these families, approximately two thirds had at least two offspring with measures of energy metabolism.

Genotypes of 503 microsatellite markers were determined at Marshfield Medical Research Foundation (Dubovsky et al. 1995), and 13 were determined at Glaxo-Wellcome, by means of fluorescent and, where necessary, radioactive labeling of primers (Schwengel et al. 1994). Median heterozygosity of markers was .68, and the sex-averaged distance between markers was 6.9 cM (median 6.4 cM). There were 22 intervals $\geqslant$15 cM without a marker, the largest being 25.6 cM on chromosome 5. Reproducibility was evaluated by duplicate typing of 75 DNA samples. Mendelian errors were checked in two stages. The distribution of marker alleles shared identical by state for each pair of siblings was analyzed for consistency with Mendelian expectations (Ehm and Wagner 1996). Probable typing errors were eliminated, marker by marker, by an algorithm that identifies family members whose elimination resolves the incompatibilities in the pedigree. Marker-allele frequencies were estimated on the basis of the remaining individuals. Marker linkage maps were created by means of these Pima data and the computer program CRI-MAP, and map distances given are according to this map (Lander and Green 1987).

Five traits were selected for analysis. Statistical tests were not adjusted for multiple trait testing. Each trait was analyzed as a residual after adjustment for appropriate covariates (see below).

Body Composition and Fat Distribution

Body composition was determined by hydrostatic weighing, with simultaneous measurement of lung residual volume (Lillioja et al. 1993). %FAT was calculated and adjusted by linear regression for the effects of age and sex. The ratio of waist circumference to thigh

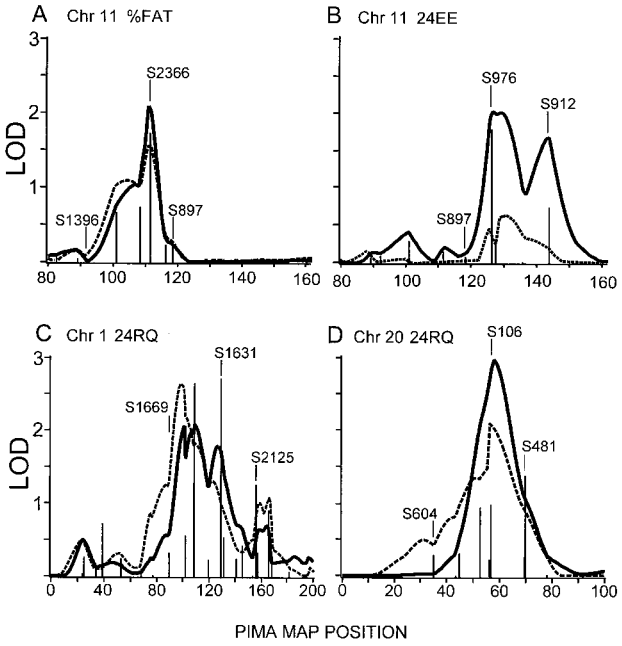

Figure 1 LOD scores for selected traits at four chromosome regions. Results from pointwise sib-pair analysis are indicated by vertical lines at the marker's map position. Unbroken lines indicate sib-pair multipoint results; and broken lines indicate sibling variance-components results. The chromosome and trait are indicated above each plot. Since the Pima map differs somewhat from published maps, the positions of a few reference markers are shown. The chromosome-number prefix (e.g., "D11" in *A*) of locus names has been deleted.

circumference ratio (W/T) was used as an indicator of fat distribution and was adjusted for age, sex, and %FAT. Although a crude indicator of fat distribution, this ratio is the only one obtainable on a large number of subjects. Since multiple measures of these traits were available for most subjects, %FAT and W/T measurements at the maximum body weight were chosen to best represent an individual's propensity for obesity. None of the subjects had diabetes at the time when these measurements were made.

Energy Metabolism

In contrast to measures of body composition and fat distribution, values at the lowest body weight were chosen for linkage analyses of energy metabolism. Metabolic predictors of body weight, such as low metabolic rate and a high respiratory quotient, tend to "normalize" in response to weight gain (Ravussin and Swinburn 1993). Thus, measures made at the lowest weight may be better indicators for the detection of genetic effects relevant to obesity. The 24-h energy expenditure (24EE) was measured in a respiratory chamber (Ravussin et al. 1986) and was adjusted for the effect of fat-free mass, fat mass, age, and sex. The sleeping metabolic rate (SMR) was calculated (Ravussin et al. 1986) between 11:00 P.M. and 5:00 A.M. (mean of all 15-min periods during which spontaneous physical activity was detected <1.5% of the time) and was adjusted for fat-free mass, fat mass, age, and sex. The ratio of carbohydrate oxidation to fat oxidation was estimated by means of the 24RQ (Zurlo et al. 1990); this variable was adjusted for the effect of energy balance during the 24-h period and for percent body fat.

Linkage Analysis

The test for linkage used quantitative-trait data and estimates or distributions of sib-pair–marker identity by descent (IBD). Sibpal 2.6 was used to test individual markers with expected IBD (pointwise linkage). Mapmaker/Sibs 2.0 was used to calculate the multipoint sib-pair IBD distribution used in sib-pair linkage analysis, by Haseman-Elston regression. One sibling was removed from a family of nine siblings, to accommodate the Mapmaker/Sibs limit of eight. Finally, a variance-components analysis (Amos 1994) was implemented that used trait data from siblings from nuclear families and the IBD sib-pair distribution from Mapmaker/Sibs 2.0. Based on the approach of specifying the genetic covariances between relatives and genetic IBD (Comuzzie et al. 1997), this method assumes multivariate normality to estimate the effect that an additive genetic locus has on a quantitative trait. Under these assumptions, variance-components methods are reported to have increased power to detect genetic effects.

Results are limited to presentation of either (*a*) map positions at which evidence of linkage, by LOD score, was >1.2 ($P < .01$) by any method or (*b*) two adjacent markers with LOD scores of 0.6–1.2 ($.05 < P < .01$) by the pointwise analysis. *P* values were converted to LOD scores by subtracting twice the *P* value from 1, taking this value's quantile from a χ^2 distribution with 1 df, and dividing by $2\log_x 10$ (Lander and Kruglyak 1995).

Empirical Evaluation of P *Values for Pointwise Linkage Tests*

P values calculated by Sibpal 2.6 using reduced df (the number of siblings in each family minus 1, summed over all families) were compared with *P* values empirically estimated by marker simulation for traits %FAT, 24EE, and 24RQ, which showed evidence of linkage (LOD $\geqslant$ 2.0; $P = .0012$). Each simulation was based on a marker with uniformly distributed alleles, the number of which was chosen to provide the same information content (calculated with Mapmaker/Sibs 2.0) at the map position for which the LOD score was maximum. This corresponded to using a 12-allele marker for %FAT, a 10-allele marker for 24EE, and 6- and 8-allele markers

Table 2

LOD Scores for Obesity Traits

	Sib-Pair Linkage Analysis					Sibling-Based Variance-Components Analysis	
	Pointwise			Multipoint			
Measure and Chromosome	Location (cM)	Marker(s)	LOD Score	Location (cM)	LOD Score	Location (cM)	LOD Score
%FAT:							
2	161.8–178.8	*D2S1776–D2S1775*	.7	159	1.1	158	1.6
2	218.7–228.7	*D2S1363–D2S427*	1.4	224	1.0	220	.7
5	104.5	*D5S1716*	1.3	105	.3	105	.6
11	111.5	*D11S2366*	1.7	111	2.1	111	1.5
14	10.4	*D14S1280*	1.3	19	1.2	20	.9
14	89.9	*D14S617*	1.2	88	.8	86	.5
17	123.7	*D17S785*	2.4	131	1.4	136	1.9
18	53.9	*D18S877*	1.6	55	1.1	57	2.3
W/T:							
1	192.4	*D1S2127*	1.2	188	1.1	188	.3
3	.0–2.0	*D3S2387–D3S1307*	2.6	0	.9	0	.6
3	72.0–84.5	*D3S3048–D3S1285*	.8	74	1.3	76	.6
3	188.4	*D3S2427*	1.2	188	1.3	188	.4
9	10.0–24.0	*D9S168–D9S925*	.7	11	1.0	10	.7
13	30.0	*D13S218*	1.7	28	.7	28	.7
13	42.1	*D13S887*	1.2	41	.5	42	.4

Note.—Pointwise and multipoint results are matched by map position where the multipoint score is the maximum. For pointwise values, only the maximum score is given when all LOD scores of contiguous markers were >1.2.

for 24RQ. To provide for an accurate estimate at the $P = .01$ level of significance, the marker was simulated 10,000 times, and the data were subsequently analyzed for linkage by Sibpal.

The accuracy of P values calculated from Sibpal was also addressed through permutation tests (Fisher 1935) using actual marker data. %FAT and 24RQ were chosen as sample traits for pointwise linkage tests. For each trait, a random-number generator was used to reassort trait values among siblings and families. The family variance was then adjusted to recreate the same average level of within-sibship variance and sib-pair correlation as existed in the actual data of the pedigrees. Thirty unlinked markers with heterozygosity of .24–.93 were selected for the simulation. The true genotypic scorings were used in tests of linkage (10,000/marker) with the randomized traits. To examine for an effect of linkage, additional simulations were done on four groups of three or four linked markers, with the between-marker distance varying from 2 cM to 8 cM.

Results

Obesity Traits

LOD scores $\geqslant 1.2$ ($P \leqslant .01$) for %FAT and W/T are shown in table 2. Regions showing linkage to %FAT did not show linkage to W/T. In general, sib-pair multipoint analysis yielded LOD scores comparable to those obtained by pointwise analysis. For %FAT, the maximum multipoint score (LOD = 2.1; $P = .001$) occurred on chromosome 11 at 111 cM (fig. 1*A*). No additional regions with LOD scores >1.2 for %FAT or W/T were detected by multipoint sib-pair analysis.

The locations of the highest LOD scores resulting from siblings-based variance-components analysis of %FAT were similar to those of sib-pair methods. The largest difference between the two methods was on chromosome 18, where the variance-components LOD score was 2.3 ($P = .0006$), which was the strongest %FAT linkage result by this method. For W/T, no additional regions with LOD scores >1.2 were detected.

Metabolic Rates

LOD scores $\geqslant 1.2$ for 24EE and SMR are shown in table 3. Although both are measures of metabolic rate, in general the pointwise LOD scores for 24EE and SMR did not correspond. By multipoint analysis, one region on chromosome 4, at 85–89 cM, showed some evidence of linkage to both traits. The maximum multipoint LOD score for 24EE occurred on chromosome 11, at 127 cM (fig. 1*B*), but is apparently distinct from the %FAT linkage result at 111 cM. LOD scores by the variance-components method were generally lower for 24EE. In fact, only one region exhibited a variance-components LOD value >1.0. The largest variance-components linkage result (LOD = 1.8; $P = .002$) for SMR occurred at 135 cM on chromosome 1.

Table 3

LOD Scores for Energy Expenditure

	Sib-Pair Linkage Analysis					Sibling-Based Variance-Components Analysis	
	Pointwise			Multipoint			
Measure and Chromosome	Location (cM)	Marker(s)	LOD Score	Location (cM)	LOD Score	Location (cM)	LOD Score
24EE:							
1	165.7–168.2	*D1S1679–D1S1677*	1.0	175	1.0	176	.7
2	9.6	*D2S1400*	1.4	0	1.3	0	1.2
2	80.9	*D2S1394*	1.5	81	.6	82	.4
4	88.5	*D4S2456*	1.4	89	.8	92	.3
11	126.3	*D11S976*	1.8	127	2.0	131	.6
18	124.0–131.4	*D18S844–D18S70*	.6	132	.8	132	.3
20	0	*D20S103*	2.8	0	1.7	0	.6
SMR:							
1	131.3	*D1S1623*	.1	13	1.0	135	1.8
1	208.2	*D1S1678*	1.6	209	.6	208	.4
4	81.9	*D4S2367*	1.9	85	1.1	84	1.2
4	164.1	*D4S1629*	2.2	164	.6	164	.5
10	6.0	*D10S1435*	1.2	6	.8	6	.5
13	104.7	*D13S763*	1.3	107	1.6	98	1.1
17	0	*D17S1308*	1.4	0	1.0	0	.5

Note.—See Note to table 2.

Respiratory Quotient

LOD scores $\geqslant 1.2$ for 24RQ are shown in table 4. The highest pointwise sib-pair LOD scores for 24RQ reoccurred on chromosome 1, at *D1S551* and *D1S1631* (LOD = 2.6; $P = .0003$; and LOD = 2.7; $P = .0002$, respectively), and on chromosome 19, at *GATA69B10* (LOD = 2.9; $P = .0001$). The two markers on chromosome 1, however, are separated by >20 cM, and a marker between them did not show evidence of linkage. On multipoint sib-pair analysis, this region of chromosome 1, at 101–126 cM, resolved into three peaks (fig. 1*C*). On chromosome 20, the multipoint LOD score was much higher than the pointwise result, with a maximum score of 3.0 at 57 cM (fig. 1*D*). Under variance-components analysis, the LOD score on chromosome 1 was higher (LOD = 2.8; $P = .0002$) and resolved into an apparently single peak (fig. 1*C*) at 99 cM. Like sib-pair analysis, this analysis showed a peak LOD score at 57 cM on chromosome 20, but the evidence was not as strong (LOD = 2.0; $P = .001$).

Empirical Estimates of False-Positive Rates

Simulations of marker genotypes, together with their analysis for pointwise linkage, resulted in the following

Table 4

Largest LOD Scores for 24RQ

	Sib-Pair Linkage Analysis					Sibling-Based Variance-Components Analysis	
	Single Marker			Multipoint			
Chromosome	Location (cM)	Marker(s)	LOD Score	Location (cM)	LOD Score	Location (cM)	LOD Score
1	101.7	*D1S550*	1.9	101	2.1	99[a]	2.8
1	108.2–108.6	*D1S1728–D1S551*	2.6	109	2.1	…	…
1	129.0	*D1S1631*	2.7	126	1.8	…	…
1	156.1	*D1S2125*	1.2	164	.7	165	1.1
3	0	*D3S2387*	1.6	2	1.1	2	1.1
5	0	*D5S392*	.4	5	1.4	1	1.3
10	0–28.3	*D10S249–D10S1412*	1.0	0	1.1	0	.7
19	32.3	*GATA69B10GATA69B10*	2.9	32	1.1	32	1.2
20	52.7–56.8	*D20S477–D20S601*	1.0	57	3.0	57	2.0
20	69.9	*D20S481*	1.4	57	3.0	57	2.0

[a] One peak was shown (see fig. 1*C*).

P-value estimates $\leqslant .01$ (LOD $\geqslant 1.2$): .0101 for %FAT, with a 12-allele marker; .0118 for 24EE, with a 10-allele marker; and .0114 and .0105 for 24RQ, with a 6- and an 8-allele marker, respectively. There is, on average, a 10% excess of false positives, at $P \leqslant .01$, between the empirical and theoretical *P* values for these traits.

Results of permutation analyses with %FAT and 24RQ were similar to the results of marker simulation. In 10,000 replicates with each of 30 actual markers, the rate of *P* values $\leqslant .01$ was 15% greater than expected (.0114 for %FAT and .0117 for 24RQ). The number of $P \leqslant .01$ that was generated by each marker is highly correlated between the two traits ($r^2 = .58$) but is not related to marker heterozygosity ($r^2 = .02$ for %FAT; and $r^2 = .04$ for 24RQ). Also, linked markers were not correlated (average $r^2 = .07$). If results from all 30 markers are pooled, a sufficient sample (300,000) can be generated for comparison of *P* values $\leqslant .001$. At this significance level, the total number of *P* values was, on average, 31% greater than expected (.00125 for %FAT and .00138 for 24RQ), indicating an increasing tendency toward the production of false positives at decreasing *P* values.

Discussion

We sought evidence of autosomal genes contributing to quantitative variation, both in obesity and in some of its underlying metabolic predictors. Sib-pair LOD scores in several regions indicate possible locations for major genes contributing to these traits: 11q21-q22 (LOD = 2.1; $P = .001$), for percent body fat; 11q23-q24 (LOD = 2.0; $P = .001$), for energy expenditure; and 1p31-p21 (LOD = 2.0; $P = .001$) and 20q11.2 (LOD = 3.0; $P = .0001$), for respiratory quotient—an idicator of nutrient partitioning. An additional region—18q21 (LOD = 2.3; $P = .0006$), for percent body fat—was revealed by a sibling variance-components method. Evidence for genetic variation contributing to these traits in these regions is not conclusive, since scores of these magnitudes are expected to occur, by chance, >5% of the time in a 5-cM genome scan (Lander and Kruglyak 1995).

There are two controversial issues involved in the interpretation of these results. First, multiple-marker analyses pose a problem in the judgment of the statistical significance of the results. Proposals have been made for characterization of LOD-score levels as "suggestive" and "significant" (Lander and Kruglyak 1995), on the basis of probabilities of occurrence at a given marker-map density. Others argue that precise *P* values cannot be derived for complex traits and propose presentation of nominal linkage values without claim as to their actual meaning (Curtis 1996; Witte et al. 1996). Given this disagreement, we have presented all LOD scores $\geqslant 1.2$ that represent nominal *P* values $\leqslant .01$. Second, we have presented *P* values calculated from all available sib pairs, which, because of nonindependence of sib pairs, some have argued is incorrect (Daly and Lander 1996). Because theoretical *P*-value thresholds are not precise, we have evaluated empirical *P* values through simulation of marker data and permutation of trait data. This empirical approach has the advantage of avoiding unrealistic assumptions about trait distribution or marker informativeness (Churchill and Doerge 1994). Because this *P*-value inflation appears to be small (~10% and ~30% at the $P \leqslant .01$ level and the $P \leqslant .001$ level, respectively), and since our strongest evidence for linkage was near the $P = .001$ level, we have chosen to present analyses based on all available siblings, which should maximize our power for detection of genetic effects.

The power of single-marker, sib-pair linkage has been tested both in simulations of hypothetical quantitative traits (Amos et al. 1989) and in our own simulations with %FAT (authors' unpublished results). Although the sample size is the largest yet reported for a genomic scan for obesity and energy metabolism, it is probably not sufficient to produce definitive evidence for genetic variability that contributes less than a majority of the variation in percent body fat, metabolic rate, or respiratory quotient. Some regions with rather modest LOD scores may indicate the presence of genes affecting obesity traits, and thus it may be useful to examine these regions, for candidate genes, in the database of human transcripts that recently has been made available at http://www.ncbi.nlm.nih.gov/SCIENCE96.

For %FAT, the strongest sib-pair–based multipoint linkage is at 11q21-q22, near *D11S2366,* and the strongest sibling-based multipoint variance-components linkage is at 18q21, near *D18S877*. There are three genes/gene clusters in the chromosome 11 interval that might be candidates for obesity. The first is a cluster of three matrix metalloproteinase genes (*MMP1, MMP3,* and *MMP8*). Proteinases such as these participate in the processing of TNFα (Edwards et al. 1996), which, when overproduced, can lead to insulin resistance and obesity in rodents (Hotamisligil et al. 1993). The second is the gene for cytosolic glycerol-3-phosphate dehydrogenase (*GPDH-C*). This enzyme and its counterpart in mitochondria play an important role in lipid synthesis. The third is the gene for ataxia-telangiectasia (ATM), which is homologous to mammalian phosphatidylinositol-3 kinases. If ATM is such an enzyme, its ability to affect insulin-stimulated glucose transport may affect obesity (Evans et al. 1995). No obvious candidate genes for obesity were found in the chromosome 18 interval.

For energy expenditure, the strongest evidence for linkage is at 11q23-q24, near *D11S976,* flanking but distinct from the %FAT linkage discussed above. Although this region borders the interval containing a se-

rotonin receptor (5-hydroxytryptamine 3 receptor), the D2 dopamine receptor, and the apolipoprotein AI and CIII genes, these genes are likely to be $\geqslant$6 cM from the peak LOD score in this region.

For the respiratory quotient, multipoint linkage results are strongest at 20q11.2, near *D20S601,* where the LOD score is 3.0, under the sib-pair method, and 2.0, under the variance-components method. In fact, in an earlier report on a smaller sample of Pima Indians, no single-marker evidence for linkage to 24RQ was found at this location (Norman et al. 1996). The location of the gene for human agouti-signaling protein (*ASIP*) is in an interval (56–60 cM on the Pima map) where the LOD score is maximum. *ASIP* is normally expressed in adipose tissue (Kwon et al. 1994; Wilson et al. 1995). Agouti's ability to block the effect of melanocyte-stimulating hormone (Lu et al. 1994; Wilson et al. 1995) has fostered speculation that agouti could either reduce lipolysis or regulate fat storage in adipose tissue (Kwon et al. 1994). If so, then agouti would represent a good candidate gene for variation in respiratory quotient. *HNFα4,* the gene that encodes hepatic nuclear factor-4α, a variant of which causes maturity-onset diabetes of the young (Yamagata et al. 1996), maps near this region of chromosome 20. Its position on the Pima map is at 65–77 cM, a region where the LOD score range is 2.0–0.5. At present, no direct connection can be made between this gene and respiratory quotient. Also in this region is *ADA,* the locus for adenosine deaminase (Rothschild et al. 1993), which can stimulate lipolysis (Heseltine et al. 1995; Tebar et al. 1996).

The next best evidence for linkage to 24RQ is at 1p22-p12, which, under multipoint sib-pair analysis, seems to show two distinct peaks, one broad area at 101–108 cM and the other at 126 cM. This region is more narrowly defined by variance-components analysis, with a single peak at 99 cM and a maximum LOD score of 2.8 (P = .0002). Within this region, two genes—*MCAD* (medium-chain acyl-CoA dehydrogenase) and *LEPR* (leptin receptor)—might be considered candidate genes. The product of *MCAD* catalyzes an early step of fatty-acid oxidation. *LEPR* is located within 1.5 Mb of *D1S198* (Thompson et al. 1997), at ~100 cM on the Pima map and near the maximum LOD score of both sib-pair analysis and variance-components analysis. The recent demonstration in Zucker fatty rats that leptin receptors mediate an increase in fatty-acid oxidation (Shimabukuro et al. 1997) suggests a mechanism for variants in *LEPR* that would affect nutrient partitioning.

Linkage to obesity in Mexican Americans has been tested, by variance-components methods, in homologues of mouse obesity genes in extended pedigrees (Duggirala et al. 1996). Positive results for chromosome 7, near the OB gene (*LEP*), have been reported with extremity skinfold thickness (LOD = 3.1) and waist circumference (LOD = 2.5), whereas LOD scores for BMI (LOD = 1.9) and fat mass (LOD = 1.2) were more modest. Although, in the region flanking the *LEP* locus, we do find a LOD score of 1.0 for waist circumference (data not shown), we cannot replicate this result for either %FAT or BMI. Other authors have reported pointwise linkages at the *LEP* locus in families selected for extreme obesity (Clément et al. 1996; Reed et al. 1996).

At 2p21, near marker *D2S1788,* in Mexican Americans, both strong evidence for linkage for plasma leptin concentrations (LOD = 4.95) and lesser evidence of linkage to fat mass (LOD = 2.75) have been reported (Comuzzie et al. 1997). Since plasma leptin concentrations are highly correlated with %FAT (Maffei et al. 1995), we examined our data from this region, for evidence of linkage to obesity. However, we find in Pima Indians no evidence, at *D2S1788,* of linkage with either %FAT or W/T.

A genomic scan in a larger sample of Pima Indians selected to be informative for type 2 diabetes reported, at the same position (in 11q21-q22) that resulted in our 2.1 LOD score for %FAT (Hanson and Pima Diabetes Gene Group 1997), a peak LOD score of 1 for BMI. However, greater LOD scores for BMI mapped nearby, at 11q22-q25, some 30 cM from the position of linkage to %FAT. This discrepancy may indicate either that BMI, derived as a measure to compare weights corrected for height, is not an accurate indicator of body composition or that the subsample of Pima families in this report is not representative of the larger sample and does not have sufficient genetic variation, in BMI, for detection of the linkage at 11q22-q25.

UCP2, a newly discovered gene for mitochondrial uncoupling protein in mice and humans, is expressed in many tissues and is an important candidate gene for obesity, because of its potential impact on metabolic rate (Fleury et al. 1997). This gene is at 11q13 in humans but is unlikely to account for the Pima linkages to either obesity or metabolic rate, since it maps 15–20 cM from the 11q21-22 linkage to %FAT and 30–40 cM from the linkage to 24-h metabolic rate. *B3AR,* the gene for B 3-adrenergic receptor, is also a candidate and has been weakly associated with resting metabolic rate in Pima Indians (Walston et al. 1995). The location of this gene, however, near microsatellite *D8S532* (Elbein et al. 1996), places *B3AR* in a region showing no evidence of linkage to either obesity or energy metabolism.

Two previous studies of obesity in Pima Indians have reported linkage of %FAT to a marker near *TNFα* (Norman et al. 1995) and to *D11S2366* (Norman et al. 1997). These studies were based on a smaller sample, comprising 88 families. If genetic homogeneity is assumed, the present, extended data should have had greater power to detect genetic variation, but linkage to the chromosome 6 region containing *TNFα* was not ev-

ident in the families in the present study. Despite our increased sample size, the LOD score for the region around *D11S2366* did not increase, and, in fact, the multipoint result was lower.

In summary, attempts to uncover, by linkage analysis, the location of major autosomal genes influencing obesity and energy metabolism have not produced definitive evidence for genetic variation in such genes. Nevertheless, several regions have shown possible evidence of linkage. Additional information about linkage in these regions is needed to help determine whether these LOD scores represent false positives or linkages to regions containing genes contributing to obesity.

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