Absence of Linkage of Obesity and Energy Metabolism to Markers Flanking Homologues of Rodent Obesity Genes in Pima Indians

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The homologues of single genes that cause obesity in rodents are suggested as candidate genes for modulation of body composition in humans. Among these genes are the four mouse mutations—diabetes *(db)*, obesity *(ob)*, tubby *(tub)*, and yellow agouti (A^y). Variation in the human counterparts to these genes (*OB*, *DB*, *TUB*, and *ASP*, respectively) may contribute to human obesity, which is thought to have a substantial genetic component. To initially assess the potential contribution of these genes to human obesity, we examined polymorphic DNA markers that, by virtue of syntenic relationships to appropriate regions of the mouse genome, should be closely linked to the human counterparts of these genes. Using combined data from 716 Pima Indians comprising 217 nuclear families, we have tested a number of polymorphic microsatellite markers (three at *DB*, two at *OB*, five at *TUB*, and three at *ASP*) for sib-pair linkage to BMI, percentage body fat, resting metabolic rate, 24-h energy expenditure, and 24-h respiratory quotient. No significant linkages were found in an analysis of all sibships or in an analysis restricted to discordant sib pairs. *Diabetes* 45:1229–1232, 1996

The role of heredity in human obesity is receiving increasing attention (1). When adiposity is assessed indirectly through BMI, a substantial portion of the variation is often attributable to genetic causes, although the estimated heritability of the effect varies widely (2). Among the strongest arguments for the inheritance of BMI are twin studies that have suggested that a majority of the variance is genetic. In contrast, an estimate of the genetic contribution to BMI variation in a study of resemblance among relatives was only 5% (3). In the same study, however, the estimate for the genetic component to percentage body fat, a better indicator of obesity, was much higher at 25%.

The prevalence of obesity among Pima Indians of the southwestern U.S. is considerably higher than in other ethnic groups (4). However, the variation in BMI among the Pima Indians is as great as the variation for age-matched groups of U.S. residents, when compared with a pooled population of white, Hispanic, and black Americans (5). Although twin-based estimates of BMI heritability in Pima Indians are not available, estimates of parent-offspring correlation (r = 0.30) and between-siblings correlation (r = 0.32) (6) support the view that genes play a role in the development of obesity in Pima Indians.

The products of the obese (*ob*) and diabetic (*db*) mouse genes are thought to be involved in the same biochemical pathway—with the *ob* protein (a secreted product of fat cells) acting to control food intake and energy expenditure (7–9) and *db* its probable receptor (10). The phenotypes of these mutant animals include hyperinsulinemia, excessive food intake, decreased energy expenditure, and lower core temperature (11,12). Because of synteny conservation between regions of mouse and human chromosomes (13), it is possible to locate and map polymorphic genetic markers near the human homologues of these obesity genes, even in the absence of the homologue's physical or genetic mapping (14). In this paper, we report our attempts, using highly informative markers mapping near obesity gene homologues, to find linkage to obesity and energy metabolism in Pima Indians through a sib-pair linkage approach for quantitative traits (15).

RESEARCH DESIGN AND METHODS

Subjects and phenotypes. Subjects are members of the Gila River Indian Community who participate in a prospective study on the development of NIDDM and its complications (16). DNA samples have been collected on 874 individuals informative for NIDDM and obesity. Excluded from linkage analyses were 45 siblings who were under the age of 18, leaving 217 families and 716 siblings available for linkage analysis. Two additional individuals were excluded as outliers for BMI. No outliers were evident for any of the other traits. The distribution of sibship size was as follows: 104 families with 2 siblings; 44 families with 3 siblings; 36 families with 4 siblings; 18 families with 5 siblings; 12 families with 6 siblings; 4 families with 7 siblings; 3 families with 8 siblings; and 2 families with 9 siblings. DNA typing data were available on both parents in 36 families and on one parent in 89 families.

BMI was calculated as weight (kilograms) divided by height (meters) squared. To reduce the effect of confounding variables, BMI was adjusted for the significant linear effects of sex, age, and date of birth. In addition, adjustments for the effects of age squared and age cubed were necessary to more fully remove the effect of age. Most of the participating individuals had biennial examinations and thus multiple measures of BMI over an average span of 16 years. To more accurately assess an individual's potential for obesity and as a partial control for weight loss associated with the development and progression of diabetes, a condi-

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RMR, resting metabolic rate.

TABLE 1
Sample sizes of traits used for linkage analyses

Phenotype	Female siblings	Male siblings	Sib pairs	Nuclear families
BMI (kg/m^2)	416	301	1,045	217
Body fat (%)	106	156	283	89
MR (kcal/day)	99	151	266	88
24-h energy expenditure (kcal/day)	65	126	180	66
24-h respiratory quotient	65	126	180	66

tion frequent in Pimas (4), the maximum recorded BMI of each individual was selected for linkage analyses. However, 154 individuals had BMI measures only when they were diagnosed as diabetic. Excluding them did not significantly alter the findings (data not shown). Percent fat was measured by hydrostatic weighing (17) and adjusted for the linear effects of age and sex. Resting metabolic rate (RMR) was determined over a 40-min interval by a ventilated hood system (18) and adjusted for fat-free mass, fat mass, age, and sex. In a respiratory chamber, 24-h energy expenditure and 24-h respiratory quotient were measured (19). The 24-h energy expenditure was adjusted for fat-free mass, fat mass, age, and sex; 24-h respiratory quotient was adjusted for energy balance, percent body fat, and sex.

Genetic markers and linkage analysis. The chromosomal localization of microsatellite markers linked to human homologues of the mouse genes are described elsewhere for *ob* and *db* (20–22) and for A^y (23). The human genomic region homologous to *tub* was mapped by identifying 23 microsatellites in the interval between HBB and PTH (24), which is the region where the *tub* mutation is located in the mouse.

Standard techniques were used for producing the genotypes of markers. Thermal cycle amplifications of genomic DNA, electrophoresis, and allele scoring are described elsewhere (25). In some cases, primer sets were rederived from published sequences (Genbank) to alter the amplified DNA product size and thus facilitate a multiplexing strategy for scoring alleles. Marker allele frequencies in the Pima population were estimated in a group selected so that none were first-degree relatives of each other. These frequencies were used to estimate identity-by-descent in the absence of fully informative parental genotypes as implemented in the SIBPAL program in the computer program package SAGE (26). In some cases, a rare allele was found segregating in one or two families but was not found in the sample of first-degree unrelated subjects. Sib-pair linkage analysis was performed using SIBPAL program (version 2.62) of SAGE (26), which accommodates a maximum of 18 alleles per locus. All traits were analyzed as quantitative, using the regression of squared sib-paired differences on identity-by-descent. To compensate for the nonindependence of siblings within sibships, this version of SIBPAL calculates P values using a reduced degree of freedom. For our dataset of purely nuclear families, this degree of freedom is simply the number of siblings in a family minus one, summed over all families. For large samples, this modification has been shown by simulation to remove the excess of false positives calculated from using the total number of sib pairs as degree of freedom (27 and R.C. Elston, personal communication).

RESULTS

For linkage analyses, phenotypes that reflected the obesity and reduced energy expenditure commonly associated with the rodent models were selected. The numbers of female siblings, male siblings, total sib pairs, and nuclear families are shown in Table 1. Allele frequencies, calculated marker heterozygosities, and approximate distances between markers for each of the rodent gene homologues are presented in Table 2.

Results of sib-pair analyses (SAGE) are presented in Table 3. At the single test significance level of 1%, there was no evidence of linkage to any trait. Data for one marker (D7S530) mapping ~1 cM telomeric of *OB* was significant ($P = 0.01$) for linkage to RMR. Another marker (HCPA2) mapping ~2 cM from *OB* on the same side (telomeric), however, was not linked. Given this lack of correspondence, and the fact that 75 linkage tests were performed, this linkage result is not considered significant. Linkage analysis for BMI was also performed on a selection of potentially more informative siblings by use of families with highly discordant pairs as suggested for improving the power of sib-pair linkage for quantitative traits (28). Selection of families with at least one sibling in the high and one in the low 20th percentile of adjusted BMI provided a test with 189 sib pairs for BMI. However, no significant linkages were found (data not shown).

DISCUSSION

Recently, human *OB* markers have been tested for linkage to NIDDM in Mexican-Americans (21). Some of these patients were tested for linkage to BMI, but no linkage was evident. The available sample, however, was quite small ($n = 77$) for detecting linkage and does not exclude a possible major effect of the human *OB* gene. With a sample size of 1,045 sib pairs, simulation suggests that an allele contributing ~30% of

TABLE 2
Frequencies of alleles for markers near homologues of rodent obesity genes in unrelated Pima Indians

	Distance	Allele frequencies							Heterozygosity
		1	2	3	4	5	6	Others	
DB markers									
D1S438	—	0.263	0.240	0.157	0.170	0.140	0.027	0.003	0.77
D1S515	1	0.540	0.110	0.093	0.077	0.067	0.057	0.056	0.65
D1S198	4	0.281	0.146	0.133	0.113	0.103	0.101	0.123	0.83
OB markers									
D7S530	—	0.343	0.325	0.205	0.075	0.022	0.007	0.023	0.78
HCPA2	1	0.302	0.298	0.129	0.089	0.097	0.028	0.057	0.71
TUB markers									
D11S1331	—	0.639	0.152	0.079	0.053	0.053	0.023	0.001	0.53
D11S1338	—	0.656	0.142	0.136	0.023	0.023	0.020	—	0.51
D11S932	3	0.523	0.167	0.103	0.061	0.047	0.035	0.064	0.69
D11S1999	1	0.507	0.149	0.149	0.076	0.073	0.024	0.022	0.69
D11S861	8	0.317	0.266	0.179	0.102	0.102	0.033	0.001	0.80
ASP markers									
D20S200	—	0.534	0.241	0.173	0.031	0.021	—	—	0.61
D20S55	2	0.622	0.182	0.059	0.038	0.035	0.024	0.040	0.62
D20S174	4	0.458	0.171	0.168	0.091	0.031	0.031	0.050	0.65

Heterozygosites are calculated.

TABLE 3
Linkage results (single-point *P* values) for markers near rodent obesity gene homologues

	BMI	Percent body fat	RMR	24-h energy expenditure	24-h respiratory quotient
DB					
D1S438	0.743	0.453	0.765	0.534	0.973
D1S515	0.917	0.234	0.491	0.218	0.790
D1S198	0.577	0.054	0.580	0.896	0.747
OB					
D7S530	0.562	0.901	0.014	0.761	0.565
HCPA2	0.525	0.971	0.282	0.785	0.512
TUB					
D11S1331	0.479	0.721	0.686	0.175	0.464
D11S1338	0.078	0.738	0.485	0.573	0.633
D11S932	0.276	0.417	0.093	0.793	0.788
D11S1999	0.552	0.891	0.241	0.362	0.514
D11S861	0.955	0.968	0.709	0.107	0.606
ASP					
D20S200	0.066	0.125	0.063	0.947	0.343
D20S55	0.501	0.361	0.605	0.383	0.477
D20S174	0.825	0.780	0.128	0.720	0.435

Results presented here are for covariate adjusted variables (see METHODS). Degrees of freedom used by Sibpal program in significance level evaluation of linkage were as follows: BMI (486), percent body fat (156), RMR (149), 24-h energy expenditure (105), and 24-h respiratory quotient (105). It is the sum over families of one less than the number of children per family.

the trait variation should be detectable with very closely linked polymorphic markers (29). It is well recognized, however, that BMI is only an indirect measure of fatness and not the most accurate indicator of obesity (30). For the other traits analyzed, percent body fat and energy metabolism, the number of sib pairs was considerably less, and an allele would have to make a much larger contribution to these phenotypes to be detected.

Our results indicate that the homologues of these rodent obesity genes, at least individually, do not account for a large proportion of the variation in BMI among Pima Indians. To a lesser extent, the data also indicate that these genes may not make a large contribution to variation in body composition or energy expenditure. Linkage results, however, cannot exclude the possibility that variants in these genes have some influence on obesity or energy expenditure. The families in this study were not selected for the obesity phenotype, and it is certainly possible that a subset of families are segregating for an allele that contributes in a major way to obesity or energy metabolism. In fact, an analysis of "affected" lean sib pairs, defined as siblings in the lower 25th percentile of BMI, showed some lower *P* values (0.004–0.02) for markers at *OB* and *TUB* (M. Devoto, personal communication), although the number of such sib pairs is very small (40).

Although not linked to obesity, a marker (D1S198) very near *DB* was reported to be linked to the acute insulin response in Pima Indians (31). If the *DB* gene, the probable *OB* protein (leptin) receptor (32), is responsible for this linkage, it may play a major regulatory role in insulin secretion but only secondarily affect obesity, and for this reason it does not appear genetically linked to either BMI or percent body fat.

Even in a well-defined and well-sampled population such as the Pima Indians, the complex nature of human obesity diminishes the chances for finding single genes that affect body size and composition. Whether or not the homologues of rodent obesity genes play some role in human obesity may be best understood by direct examinations of these genes for coding and regulatory sequence variation. The recent cloning of the *OB* (33), *OB* receptor (32)—the *db* homologue—and ASP (23) genes permits searches for molecular variants that may affect obesity and metabolism. A recent study found no coding region variants for *OB* in five obese and five lean subjects (34). At present, no *OB* coding region variants have been detected in Pima Indians or other morbidly obese subjects (35).

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