Brief Genetics Report

An X-Chromosome Scan Reveals a Locus for Fat Distribution in Chromosome Region Xp21–22

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Several groups have completed autosomal genome scans for human obesity, but only two have examined the X chromosome. A French group reported linkage of BMI to Xp and Xq markers, and a Finnish group reported linkage of BMI to Xq. We scanned the X chromosome in two cohorts, 190 European-American families (940 members) and 43 African-American families (208 members). We examined five correlated obesity phenotypes, BMI, body fat percentage, hip and waist circumferences, and plasma leptin concentration. We also examined leptin resistance (leptin/BMI) and fat patterning (waist-to-hip ratio [WHR]). Variables were adjusted for age within generation, race, and sex. We genotyped 20 markers with average spacing of 10 cM and no interval >22 cM and conducted nonparametric analyses. Suggestive linkage was found for WHR only. Linkage was supported in both family sets, and support was especially strong for females. *Z* scores for analyses of female phenotypes were 2.69, 1.73, and 2.37 (*P* = 0.0036, 0.0418, and 0.0089) for African-Americans, European-Americans, and the combined sample, respectively. The peaks were 51–73 cM from the p terminus, 14–34 cM distal of the French report in Xp22. Our results suggest that a quantitative trait locus influencing fat distribution in women may lie in chromosome region Xp21–22; however, the linked interval is large and differs substantially from that of the French and Finnish groups. Given the positive but divergent results, it would be worthwhile for others to examine the X chromosome. *Diabetes* 51:1989–1991, 2002

Obesity is a common, multigenic trait that conveys an increased risk for several diseases, especially type 2 diabetes, cardiovascular disease, and hypertension. Several groups (1) including our own (2) have completed autosomal genome scans aimed at detecting linkage to obesity-related phenotypes. Only two groups have completed scans of the X chromosome, however. A French group (3) found linkage to a marker in chromosome region Xp22 and Xq, and a Finnish group (4) reported linkage to a marker in Xq24. Both studies used qualitative thresholds of BMI as the obesity phenotype.

In the current study, we conducted a scan of the X chromosome in two sets of families having both extremely obese (BMI $\geq$40 kg/m^2) siblings and normal weight (BMI $<$27 kg/m^2) siblings and parents. One sample included 190 European-American families having 940 individuals; the other included 43 African-American families having 208 individuals. Family members were genotyped for 20 microsatellite markers with an average spacing of 10 cM. We examined four overlapping qualitative phenotypes—BMI $\geq$27, $\geq$30, $\geq$35, and $\geq$40 kg/m^2—and five correlated obesity-related phenotypes—BMI, body fat percentage, waist circumference, hip circumference, and plasma leptin concentration. We also examined the waist-to-hip ratio (WHR) as a measure of fat patterning and the ratio of leptin to BMI as a measure of leptin resistance. All quantitative variables were adjusted for age within generation, sex, and race. We conducted nonparametric linkage (NPL) analyses using the computer programs Genehunter (for the qualitative phenotypes) and Mapmaker Sibs (for the quantitative phenotypes). Multipoint linkage analyses were conducted separately in the two samples and in a combined sample. The results are summarized in Table 1.

The WHR reached nominal statistical significance in the combined sample, and the results were strongest for women. Separate analyses of the two samples gave nominally significant results for European-American women and for African-American women. The analysis of the combined sample gave a *Z* value of 2.01 at 49 cM. When the analysis was restricted to phenotypes of women, the *Z* value increased to 2.37 and shifted to 69 cM. Within the individual samples, the *Z* values were 2.69 at 73 cM for African-American women and 1.73 at 51 cM for European-American women. Multipoint results are summarized in Fig. 1.

Only one of the analyses of the qualitative variables yielded marginally significant results: the Genehunter analysis of the phenotype BMI $\geq$40 kg/m^2 yielded a *Z* score of 1.18 at 47 cM.

Empirical *P* values derived from simulations support the reliability of our major findings. For the female-only sample for which male phenotypes (but not genotypes) were treated as being unknown, only 1 of 100 replicates (P = 0.01) had a *Z* score equal to or greater than the observed value of 2.37. For the combined analyses (male and female

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LDB, Genetic Location Database; NPL, nonparametric linkage; WHR, waist-to-hip ratio; WI, Whitehead Institute for Biomedical Research/MIT Center for Genome Research.

TABLE 1
Multipoint linkage results for obesity-related phenotypes and X chromosome markers

Sample	Phenotype	NPL or *Z* score	Location
Combined sample	BMI ≥40 kg/m^2	1.18	47 cM
Combined sample	WHR	2.01	49 cM
Combined sample: women only	WHR	2.37	69 cM
African-Americans: women only	WHR	2.69	73 cM
European-Americans	WHR	1.85	44 cM
European-Americans: women only	WHR	1.73	51 cM

phenotypes included), only 2 of 100 ($P = 0.02$) replicates had a Z score ≥2.01.

Our results are most similar to those of the French group, who reported suggestive linkage of BMI to a marker at ~37 cM; however, the linked intervals are not overlapping. Our own results span a large region of Xp from 47 to 73 cM. The most significant results were for a measure of body fat distribution, but there were marginally significant results within the same region for BMI.

It is interesting that the phenotype linked to the X chromosome in our study is a measure of body fat distribution that has a low correlation with overall adiposity, at least in these extreme samples. Segregation studies of obesity phenotypes have not suggested a major role for sex-linked genes (5). However, fat patterning is certainly different in men and premenopausal women. In fact, the overall heritability appears to be somewhat higher in women than in men in both our samples. The heritability of WHR based on these samples was as follows: combined males and females, 0.415; combined females, 0.554; European-American males and females, 0.361; European-American females, 0.575; African-American males and females, 0.399; and African-American females, 0.485. We did not estimate heritability separately in men because of the relatively small samples.

In the past, WHR has been used as an index of fat patterning to distinguish between upper and lower body fat, specifically whether fat tended to be stored in the hips and thighs as opposed to the abdomen. The measure was found to be a risk factor for diabetes (6) and cardiovascular disease (7), through its imperfect relationship with abdominal fat. In recent years, other measures have emerged that appear to be better indexes of the amount of abdominal fat, particularly waist circumference (8). In our own sample, having an extreme range of phenotypes from BMI 16 to 86 kg/m^2, waist circumference is highly correlated with BMI ($r = 0.83$) and is thus tied to overall obesity. WHR therefore reflects a pattern of fat distribution that is largely independent of overall obesity in our sample ($r = -0.13$).

Given the broad interval within Xp, it is not possible to suggest specific candidate genes. However, at least two genetic syndromes with obesity as a feature map to this region, MEHMO (mental retardation, epileptic seizures, hypogonadism and -genitalism, microcephaly, obesity) syndrome and Simpson-Golabi-Behmel syndrome (1). Quantitative trait loci have also been mapped to this region, one for body weight in mice and another for back-fat thickness in swine (1).

Overall, our results provide suggestive evidence of linkage of a measure of fat patterning, the WHR, to markers in human chromosome region Xp21–22. Further study and independent replication are needed to establish the reliability of the findings.

RESEARCH DESIGN AND METHODS

Details of family recruitment have been reported previously (2,9), and all procedures have been reviewed by the University of Pennsylvania Committee on Studies Involving Human Beings. Briefly, families were selected through an index case with BMI ≥40 kg/m^2. In addition, at least one obese sibling (BMI ≥30 kg/m^2), one normal-weight (BMI <27 kg/m^2) sibling, and one normal-weight parent were required. The families included in analyses for this report were 190 non-Hispanic Caucasian (European-American) families having 940 individuals and 43 African-American families having 208 individuals. On average, parents were in their middle sixties and offspring in their late thirties. The European-American sample ranged from 15 to 90 years old (average 47 ± 15) (±SD). The African-American sample ranged from 18 to 86 years old (average 44 ± 14). Average BMIs for African-American fathers, mothers, sons, and daughters were 28 ± 5, 35 ± 10, 30 ± 8, and 39 ± 9, respectively. Average BMIs for European-American fathers, mothers, sons, and daughters were 29 ± 6, 33 ± 9, 32 ± 8, and 39 ± 12 kg/m^2, respectively.

BMI was based on measured height and weight (weight [kg]/height [m]^2). Qualitative phenotypes included BMI ≥27, ≥30, ≥35, and ≥40 kg/m^2. Quantitative phenotypes included BMI, body fat percentage based on bioelectric impedance (Valhalla Scientific), waist circumference, hip circumference, WHR, and plasma leptin concentration based on an average of duplicate radioimmunoassays (Linco Research, St. Charles, MO). A ratio of leptin to BMI was used as an index of leptin resistance.

All quantitative variables were residualized for linear effects of age within generation (parent versus sibling), sex, and race. Higher-order age effects were not significant.

We typed a total of 20 markers spanning chromosome X, with an average spacing of 10 cM and an interval size of 0.9–22 cM. The markers and approximate cM location from the p terminus were DXS7100 (7.8), DXS8022 (24.2), DXS451 (37.1), DXS1061 (41.7), DXS997 (47.0), DXS8113 (56.6),

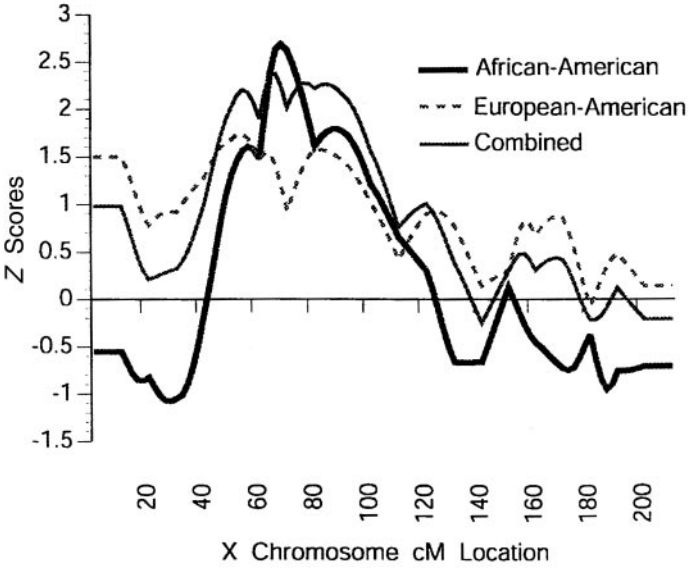

FIG. 1. NPL scores for linkage of WHR in women with chromosome X markers in samples of European-American, African-American, and pooled families.

DXS1003 (77.0), DXS991 (86.9), DXS1199 (87.8), DXS981 (89.7), DXS441 (96.0), DXS1002 (97.4), DXS454 (107.4), DXS1191 (117.4), DXS1059 (121.0), DXS1212 (143.0), DXS8094 (153.7), DXS8073 (174.7), DXS8061 (193.8), and DXS8087 (198.1). DNA amplification was performed by means of a PTC100 thermocycler (MJ Research, Waltham, MA). PCR was conducted in a 10-μl reaction volume under conditions appropriate for each marker (available on request). PCR primers were labeled with [^{33}P]ATP, and products were separated by PAGE. Band patterns were independently scored by two individuals blind to phenotype. All genotypes were checked for Mendelian inheritance using the computer program Genehunter and either resolved or recoded as unknown. Any genetically unrelated parents and siblings were excluded, as were all half-siblings.

NPL analyses for qualitative traits were performed using Genehunter, version 1.3 (10). Sibship size ranged from 2 to 10 in European-American families and from 2 to 8 in African-American families. Both sets of families had a median sibship size of 3. Analyses of quantitative phenotypes were performed using Mapmaker SIBS, version 2.0 (11). All possible sibling pairs were included, weighting sibships with multiple pairs by $\Sigma(N - 1) - 2$ (12). For Mapmaker analyses, four extended families were split into two sibships each, and one family was split into three sibships. Family branches with single sibs were eliminated. Heritability of WHR was computed using the polygenic model in the beta version of the computer program Solar (http://www.sfbr.org/sfbr/public/software/solar/index.html).

We focused primarily on nominal P values because of the history of the sample. Eighty-three European-American and nine African-American families were included in the initial autosomal scan, and somewhat different phenotypes were examined for the X scan. We were able to devise no simulation model that accurately reflected the complex history of analyses of the two samples. Based on previous simulations, there will be a high proportion of false-positive results at marginal significance levels of $P < 0.05$ but a decreasing proportion of errors at more stringent levels—e.g., beyond $P < 0.01$.

We conducted simulations to obtain empirical P values for the major findings only. Specifically, we used the computer program Simulate (13) to generate 100 replicates of marker genotypes for the sample using the WHR phenotype, family structures, marker map, marker allele frequencies, and marker information content from our sample data. The sample replicates were analyzed using the NPL model in Mapmaker Sibs. Replicates having multipoint Z scores greater than or equal to the value observed for the analyses of real data were counted and divided by 100 to obtain empirical P values.

In the African-American sample, one or both parents were participating from 39 of 53 families (91%; 33% with two parents, 58% with one parent), and there were 156 siblings, yielding a total of 256 pairs. The females-only analysis of WHR had a total of 121 pairs with valid observations. In the European-American sample, one or both parents were participating from 183 of 190 families (96%; 46% with two parents, 50% with one parent), and there were 670 siblings, yielding a total of 994 pairs. The females-only analysis of WHR had a total of 357 pairs with valid observations. To guard against a possible influence of outliers in the phenotype distributions, we report only NPL scores from Mapmaker Sibs, which are based on rank data.

Gene frequencies were estimated by allele counting using all individuals who provided DNA. This approach gives asymptotically unbiased estimates of the allele frequencies (14). Frequencies were estimated separately for the European-American, African-American, and combined samples. Because of the large sample sizes, all loci had significant between-sample differences in the frequencies of some alleles. However, the magnitude of the differences was generally small, and the rank order of frequencies for the alleles tended to be the same, with minor exceptions. In any case, using gene frequency estimates derived from the combined sample should have little impact on estimates of identity by descent, because parental genotypes were available for most families. With 75% average heterozygosity and a median sibship size of 3, the information from a single-parent family averages ~87% of that from two-parent families.

Map locations for markers were taken from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (WI) (http://www-genome.wi.mit.edu). Markers not found in the WI database were placed using the Genetic Location Database (LDB) (http://cedar.genetics.soton.ac.uk/public_html/ldb.html) and the Genome Database (http://gdbwww.gdb.org). Initially, all markers were chosen to be 10 cM apart based on LDB. When we derived the map using the approach in our original genome scan, some of the distances changed, because the WI map depends more on physical distance. Since locus order did not change, there should be little effect on results, and additional analyses using the LDB map gave essentially the same Z scores.

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