Brief Genetics Report

Linkage for BMI at 3q27 Region Confirmed in an African-American Population

Amy Luke,[1] Xiaodong Wu,[1] Xiaofeng Zhu,[1] Donghui Kan,[1] Yan Su,[2] and Richard Cooper[1]

Significant and suggestive linkage for BMI on 3q27 has been reported by several groups, including our own study in African Americans. To further establish the linkage evidence on 3q27, we recruited an independent African-American sample comprising 545 individuals in 128 families. We genotyped 15 short tandem-repeat markers evenly spaced in the 112 cM region around the peak on 3q27 identified in our earlier study. Multipoint linkage analysis by GENEHUNTER2 gave the maximum logarithm of odds (LOD) score 2.4 at map position 188 cM in this sample. When we combined the two samples, linkage evidence was increased to a maximum LOD score (MLS) of 4.3 (point-wise $P = 4.34 \times 10^{-6}$) at 188 cM, with a 7 cM 1-LOD-drop interval around the peak. The multiple replications of linkage evidence in the region on 3q27 strongly confirm its potential importance as a candidate region in the search for obesity-related genes. *Diabetes* 52:1284–1287, 2003

Genome-wide linkage analyses for obesity have now been reported for European descendants (1–8), Mexican Americans (9,10), Pima Indians (11–13), and African Americans (14). Evidence of linkage has emerged for several chromosomal locations (15,16), with at least six regions significantly linked to an obesity phenotype (maximum LOD score [MLS] > 3.3) and replicated in at least two studies based on the recent Human Obesity Gene Map (16). Despite these positive results, the literature contains inconsistent and contradictory reports. Consistent replication is the logical escape from this dilemma, although this is difficult to achieve as linkage is generally underpowered, and even quantitative trait loci (QTL) with strong effect are not expected to generate significant evidence of linkage consistently (17).

We previously reported suggestive evidence that BMI was linked to D3S2427 on chromosome 3q27 (map location = 187 cM, LOD score = 1.8) in an African-American sample (14). At the time, our findings provided replication of linkage at this location for several obesity phenotypes as reported by Kissebah et al. (1). Linkage with early-onset diabetes was also found on chromosome 3q27 (18). Our earlier report was the result of a genome-wide scan study for obesity and hypertension as part of the Family Blood Pressure Program (19). To confirm our findings at the 3q27 region, we conducted a linkage analysis in a separate African-American sample of families and again by combining the new sample with that used in our original report on 3q27.

RESEARCH DESIGN AND METHODS

Recruitment of participants. Two samples of African Americans were included in this analysis. The total sample is composed of 1,163 individuals from 330 families. Sample 1 included 618 individuals from 202 families, as reported previously (14). The second independent sample (sample 2) included 545 individuals from 128 families. While sample 1 was recruited based on a proband between the ages of 25 and 45 years and a blood pressure in the upper 25th percentile of the age-sex–specific distribution for this community, sample 2 was recruited with only the age restriction for the proband. After identification of the proband, all available nuclear family members were asked to participate. No restrictions were imposed in terms of weight or diet history in either sample. Our previous genome-wide scan for obesity was performed in sample 1 (14).

A clinical examination was performed that included medical history, anthropometric measurements, and blood collection. Weight was measured in light clothing on a calibrated electronic scale, and height was measured using a stadiometer.

Genotyping. The genotyping procedure for sample 1 was described in detail elsewhere (14). Briefly, 387 microsatellite markers (average 1 marker/10 cM) were genotyped at the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotyping Service, Marshfield, Wisconsin, in sample 1. To further establish the evidence for linkage at 3q27 region from sample 1, we identified and genotyped 15 microsatellite markers in sample 2 in a 112-cM region around the linkage peak at this region. The mean heterozygosity for these 15 markers was 0.78 with an average sex-equal distance of 7.5 cM between two flanking markers. Of these 15 markers, nine markers were located on a 43-cM region around the region showing the strongest evidence of linkage in our original genome scan analysis, with an average distance of 4.8 cM (14). There were a total of 21 markers in the combined samples; 8 markers were genotyped in both samples. Because the samples were genotyped in two different labs, systematic allele shifting was adjusted for these markers according to the allele frequency distributions in the samples. We further calculated the identity-by-descent (IBD) sharing in the two samples separately without adjusting for the allele shifting. This IBD matrix was then used in the linkage analysis for comparison to the original results. In addition, we tested for linkage after setting the six markers with shifted alleles to missing in both populations. RELCHECK (20) was used to check the biological relationships within the pedigrees based on the available markers; it classified the biological relationship using the likelihood method. Because of the limited number of markers, the rates of misclassification could be high and large numbers of true sibpairs could be discarded (21). Therefore, the reported family relationships were conserved unless extreme statistical significance was identified. PEDCHECK (22) was used to check for Mendelian inconsistency. Errors identified by PEDCHECK that were not based on misclassification of pedigrees were

From the [1]Department of Preventive Medicine and Epidemiology, Loyola University Stritch School of Medicine, Maywood, Illinois; and the [2]Department of Pathology, Loyola University Stritch School of Medicine, Maywood, Illinois.

Address correspondence and reprint requests to Amy Luke, Department of Preventive Medicine & Epidemiology, Loyola University Stritch School of Medicine, 2160 South First Ave., Maywood, IL 60153. E-mail: aluke@lumc.edu.

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IBD, identity by descent; LOD, logarithm of odds; MLS, maximum LOD score; QTL, quantitative trait loci.

TABLE 1
Descriptive characteristics of participants

	Sample 1		Sample 2		Combined sample	
	Men	Women	Men	Women	Men	Women
Individuals (*n*)	250	368	193	352	443	720
Families (*n*)	201		128		329	
Sibpairs (*n*)	311		271		582	
Half-sibpairs (*n*)	212		84		296	
Age (years)	38.6 ± 10.1	41.8 ± 12.4	35.3 ± 14.8	36.7 ± 5.1	37.1 ± 12.5	39.2 ± 14.0
Height (cm)	176.6 ± 7.2	164.0 ± 7.2	175.9 ± 9.0	164.3 ± 7.1	176.3 ± 8.0	164.1 ± 7.2
Weight (kg)	84.4 ± 21.4	87.2 ± 24.0	82.7 ± 21.7	83.9 ± 23.2	83.7 ± 21.5	85.6 ± 23.7
BMI (kg/m^2)	27.0 ± 6.6	32.5 ± 8.8	26.5 ± 6.0	31.0 ± 8.3	26.8 ± 6.3	31.7 ± 8.5
BMI ≥30 (%)	25.2	54.0	24.6	48.3	24.9	51.2

Data are means ± SD unless otherwise indicated.

assumed to have occurred in the genotyping process, and the associated markers were set to missing among the appropriate family members.

Statistical analysis. Linkage analysis of BMI was performed using the multipoint variance component method implemented in GENEHUNTER2 (23). The variance component method specifies the expected genetic covariances between relatives as a function of their IBD relationships at a marker locus. The IBD probabilities were estimated from all available genotyped marker loci. The likelihood ratio test was applied to test the null hypothesis of no additive genetic variance due to a QTL. Sex, age, and age squared were incorporated as covariates, and their effects were simultaneously estimated by the maximum-likelihood method. Allele frequencies were estimated from the marker data using all of the individuals. Marshfield map distances were used in the linkage analysis. Linkage analysis was performed first in sample 2 and also repeated in sample 1. We then performed linkage analysis using the combined data of the two samples. To make the allele calling consistent in the two samples, we checked the allele frequency distribution for both samples and adjusted any allele shifting before combining them for further analyses.

To assess the region-wide empirical significance of our result, we performed a simulation study in the combined sample. We retained the pedigree and phenotype data and simulated the marker genotype data based on the observed marker allele frequencies. One thousand replications were generated and analyzed by GENEHUNTER2.

RESULTS

The descriptive characteristics of the participants are presented in Table 1. Women were on average slightly older than men and more likely to be obese in both samples. The participants in sample 1 were somewhat older and heavier than those in sample 2. The heritability for BMI was 53% in the combined sample after adjusting for sex, age, and age squared calculated by GENEHUNTER2.

The multipoint linkage results for BMI from GENEHUNTER2 in sample 1, sample 2, and the combined sample at 3q27 region are presented in the figure. The MLS = 2.4 for new sample 2 was found at 188.29 cM, at marker D3S2427, with 1-LOD drop interval from 180 to 196 cM. The maximum LOD score (MLS = 2.0) was found at 187.51 cM, between maker D3S3676 and marker D3S2427 in sample 1. The 1-LOD drop interval was from 172 to 192 cM. The MLS is little higher than the original result (MLS = 1.8) (14) in sample 1 because more genotype cleaning was performed and a new version of GENEHUNTER software was used in this analysis. Theoretical analysis and a simulation study show that a 1-LOD-drop support interval corresponds roughly to a 90% confidence region in the case of a dense map of markers and provides even greater probability than a support region of coverage for sparser maps (24). In the combined sample, the MLS = 4.3 was found at 187.65 cM between marker D3S3676 and D3S2427 (unadjusted point-wise $P = 4.3 \times 10^{-6}$), with 1-LOD drop interval from 185 to 192 cM. The distance between MLS peaks between samples 1 and 2 was <1 cM, and most of the 1-LOD drop intervals overlapped the other. The MLS for the combined sample was close to the sum of the MLS for the two samples at the same location. When we used the IBD matrix calculated in the two samples separately without adjustment for allele shifting, the results were the same. In addition, there was only a modest effect on the results of linkage analysis after setting the six markers with shifted alleles to missing: the MLS was decreased from 4.3 to 4.1 at 187.65 cM. The variance accounted for by this QTL was estimated at ~40%. We recognize, however, that the strength of the QTL-specific effect is almost certainly overestimated by this method of analysis (25).

The region-wide empirical *P* value was calculated based on 1,000 replicate data sets for the combined sample. We found only two replicates having MLS >4.3 across the 100-cM region, which gave a region-wide empirical *P* value of 0.002.

DISCUSSION

In a combined analysis of two samples of African-American families, we found a highly significant result for linkage with BMI at a region on 3q27 (185–192 cM, MLS = 4.3). In each of the two samples separately, suggestive linkages (MLS = 2.0 and MLS = 2.4) were observed at the same region. Encouragingly, at least another four studies have reported significant linkage at the same region.

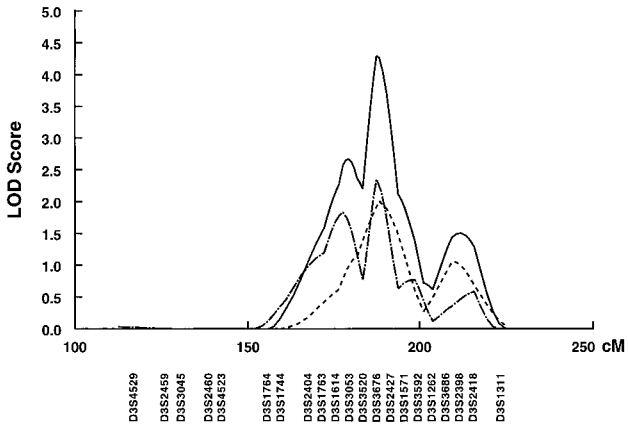

FIG. 1. Multipoint linkage results for BMI at the chromosome 3 region (112.42–224.8 cM) in sample 1 (- - - -), sample 2 (—·—), and the combined sample (—).

Kissebah et al. (1) reported strong evidence for linkage for several obesity-related phenotypes (LOD from 2.4 to 3.5) at the same region (182–200 cM) in 2,209 individuals from 507 nuclear Caucasian families. Vionnet et al. (18) also report significant linkage with diabetes or glucose intolerance in French persons aged 45 years and older. Not only have we previously reported suggestive linkage on 3q27 in sample 1 (14), we also reported the results of a combined analysis of genome scans for obesity using the interim data from the Family Blood Pressure Program (26). Using data from 6,849 individuals from four ethnic groups (European American, African American, Mexican American, and Asian), we found strong linkage evidence for a QTL at 3q27 for BMI (marker D3S2427, LOD = 3.4) (26). This large study further demonstrates that the linkage evidence on 3q27 is unlikely to be a false positive.

Several potential candidate genes have been identified around this region, including adiponectin (APM1), which is ~10 cM away from our peak, glucose transporters (GLUT2), and apolipoprotein D (Apo-D) (15). Among these genes, adiponectin gene (3q27) seems the most interesting. APM1 is the most abundant gene transcript specific to adipose tissue (27). Although the precise function of the adiponectin in energy expenditure and fat partitioning remains unclear, it is known that its circulating level is inversely correlated with BMI (28) and its mRNA level is suppressed in adipose tissue of obese animals and humans (29). Several studies have recently shown that polymorphisms in APM1 are associated with type 2 diabetes and other features of the insulin resistance syndrome (30–33). However, it is still not clear that the linkage peak identified in this region is due to the effect of APM1, and further studies should be warranted.

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