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Short communication

A locus affecting obesity in human chromosome region 10p12

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Abstract

Aims/hypothesis. Obesity is a complex trait influenced by multiple genes. We evaluated linkage in three regions of human chromosome 10 previously linked to obesity-related phenotypes.
Methods. We conducted non-parametric linkage analysis of obesity-related phenotypes in cohorts of 170 European-American and 43 African-American families having extremely obese and normal weight subjects.
Results. We found support for linkage of an obesity phenotype (BMI ≥ 27 kg/m^2) in both cohorts, as well as in a combined analysis (European-American cohort, Z = 1.90, $p = 0.03$; African-American cohort, Z = 2.25, $p = 0.014$; combined cohort, Z = 2.55, $p = 0.005$).
Conclusion/interpretation. These results confirm previous reports of linkage in French and German families. The consistency of results across these four cohorts supports the localization of a quantitative trait locus influencing obesity to human chromosome region 10p12. [Diabetologia (2001) 44: 363–366]

Keywords Obesity, genetics, linkage, body mass index , chromosome 10, complex trait, African-American, European-American.

Obesity is a multigenic trait that is a major risk factor for a number of common diseases, particularly Type II (non-insulin-dependent) diabetes mellitus, cardiovascular disease and hypertension. A recent review reported a total of 44 genomic locations with possible linkage to one or more obesity-related phenotypes [1]. Because false positive findings are common in genome scans for complex traits, replication is essential before undertaking gene identification studies. Previously, linkages for obesity traits have been replicated in three regions, 2p21, 7q31, and 20q13 [1].

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Abbreviations: PCR, Polymerase chain reaction; IBD, identity by descent; NPL, non-parametric linkage.

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To date one of the strongest findings of linkage was reported by French investigators, linking obesity (BMI ≥ 27 kg/m^2) to markers in chromosome 10p12 [2]. Recently [3], these results were replicated in a cohort of German families. In our previous genome scan [4], we did not find significant linkage to this region, although we did find secondary linkages for centromeric and distal 10q markers, corresponding to secondary peaks found in the French study for a leptin phenotype. Our genome scan had a relatively small sample size of only 92 families and we examined more extreme levels of obesity (BMI ≥ 30 kg/m^2 and BMI ≥ 40 kg/m^2) and we did not have dense marker coverage in the region of the French linkage finding.

In the current analysis, we examined linkage in this region in cohorts of 170 European-American and 43 African-American families (including the 92 families from the original genome scan).

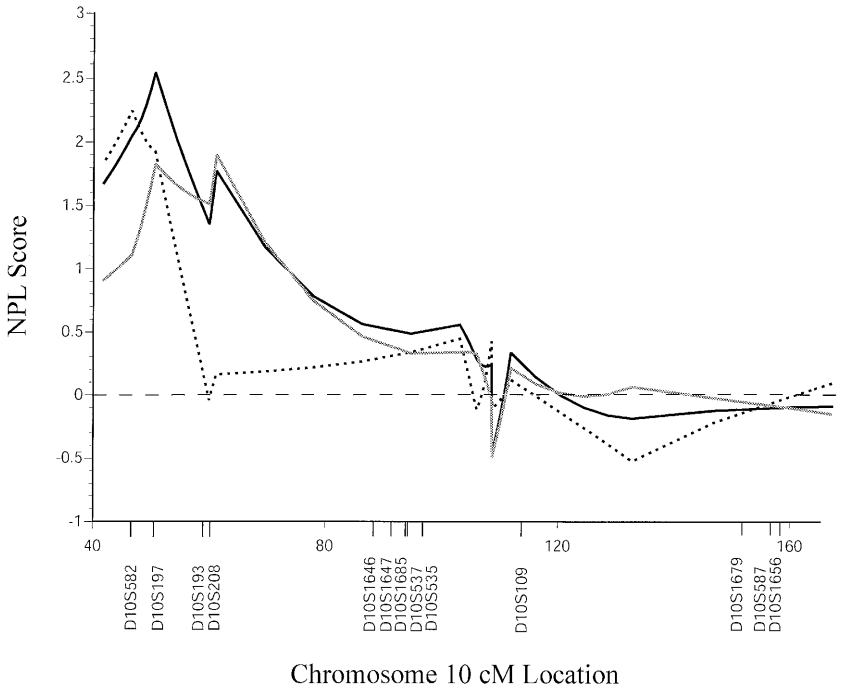

Fig. 1. Non-parametric linkage (NPL) scores for linkage of an obesity phenotype (BMI ≥ 27) with chromosome 10 markers in cohorts of European-American, African-American and pooled families. The solid line refers to the combined sample (——), the dashed line to the African-American sample (- - - -), the shaded line to the European-American sample (▒▒▒)

Subjects and methods

We recruited families as reported previously [4, 5]. All procedures were reviewed by the University of Pennsylvania ethics committee. Briefly, families with a body mass index (BMI) greater than or equal to 40 kg/m^2 were selected through an index case. In addition, at least one obese sibling (BMI ≥ 30 kg/m^2), one normal weight sibling and one normal weight parent (BMI < 27 kg/m^2) were recruited. We analysed 170 non-Hispanic Caucasian (European-American) families comprising 862 subjects and 43 African-American families comprising 212 subjects. Parents were, on average, in their middle sixties and offspring in their late thirties. Average BMI for African-American fathers, mothers, sons and daughters was 30 ± 6, 33 ± 9, 32 ± 8, and 39 ± 12, respectively. The average BMI for European-American fathers, mothers, sons and daughters was 28 ± 5, 35 ± 10, 30 ± 8, and 39 ± 9 kg/m^2, respectively.

The BMI was based on measurements of height and weight (BMI = weight(kg)/height(m)^2). Qualitative phenotypes included BMI of more than or equal to 27 kg/m^2 and a BMI of more than or equal to 30 kg/m^2. Quantitative phenotypes included BMI, percent fat based on bioelectric impedance (Valhalla Scientific), a ratio of waist-to-hip circumferences and plasma leptin concentration based on an average of duplicate radioimmunoassays (Linco Research, St. Charles, Mont., USA). All quantitative variables were residualized to find the linear effects of age within generation (parent vs sibling), sex and race. Higher order age effects were not statistically significant.

We typed a total of 13 markers on chromosome 10, including 4 markers from 10p (D10S582, D10S197, D10S193, D10S208), 6 centromeric markers (D10S1646, D10S1647, D10S1685, D10S537, D10S535, D10S109) and 3 from distal 10q (D10S1679, D10S587, D10S1656), locations that correspond to previous peaks reported by the French and by our earlier scan. A DNA amplification was carried out by means of a PTC100 thermocycler (MJ Research, Waltham Mass., USA). We conducted PCR in a 10 μl reaction volume under conditions appropriate for each marker (conditions available on request). The PCR primers were labelled with ^{33}P ATP and PCR products were separated by polyacrylamide gel electrophoresis (PAGE). Band patterns were independently scored by two subjects 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.

Non-parametric linkage analyses for qualitative traits were done using the computer program GENEHUNTER Version 1.3 [6]. Sibship size ranged from 2 to 10 in European-American families and from 2 to 8 in African-Americans. Both sets of families had a median sibship size of 3. The analysis of quantitative phenotypes was carried out using the computer programme MAPMAKER SIBS Version 2.0 [7]. All possible sibling pairs were included, weighting sibships with multiple pairs by Σ(N-1)-2 [8]. For MAPMAKER analysis, 4 extended families were split into two sibships each and one family was split into three sibships. Family branches with single sibs were eliminated. The European-American cohort contained a total of 894 pairs. Pairs with valid observations ranged from 592 for percent fat, 622 for leptin, and 691 for waist-to-hip ratio to 876 for BMI. The African-American cohort contained a total of 256 pairs. Pairs with valid observations ranged from 200 for percent fat, 225 for leptin, and 214 for waist-to-hip ratio to 250 for BMI. We report only the non-parametric linkage (NPL) scores for MAPMAKER SIBS, which are based on rank data in order to guard against a possible influence of outliers in the skewed phenotype distributions. Gene frequencies were estimated by allele counting using all subjects who provided DNA. This approach gives asymptotically unbiased estimates of the allele frequencies [9]. Frequencies were estimated

separately for the European-American cohort, the African-American cohort, and for the combined cohorts. Using gene frequency estimates from the combined samples should have little impact on estimates of identify by descent, because parental genotypes were available for most families. One or both parents were available in 94% of European-American families and in 95% of African-American families. Map locations for markers were taken from the Whitehead Institute for Biomedical Research/Massachusetts Institute of Technology 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 (http://cedar.genetics.soton.ac.uk/public_html/ldb.html) and the Genome Database (http://gdbwww.gdb.org/gdb/gdbtop.html).

An exploratory association analysis was carried out using the transmission disequilibrium test as implemented in GENEHUNTER 2 [6].

Results

The results for subjects with a body mass index greater than or equal to 27 phenotype reached nominal significance for the European-American cohort ($Z = 1.90$, $p = 0.03$), the African-American cohort ($Z = 2.25$, $p = 0.014$), and the combined cohort ($Z = 2.55$, $p = 0.005$) (Fig. 1). Results for subjects with a body mass index greater than or equal to 30 did not reach statistical significance, nor did the quantitative measures for BMI, percent fat and plasma leptin. The peak non-parametric linkage (NPL) score for the combined cohort is at D10S197. For the European cohort, the peak NPL score is about 10 cM centromeric near marker D10S208 and for the African-American cohort, the peak NPL score is about 4 cM telomeric near marker D10S582. A secondary peak was observed for the European-American cohort at 4 cM ($Z = 1.83$, $p = 0.03$) at marker D10S197. The two peaks for the European-American cohort correspond almost exactly to those reported previously in the French (D10S197) and German (D10S208, which is between D10S193 and TCF8) cohorts.

We also carried out exploratory linkage disequilibrium analysis using the transmission disequilibrium test implemented in GENEHUNTER 2 and triads within the same families included in the linkage analysis. Because of the small size of the African-American cohort, we restriced these analyses to the European-American families. We examined individual alleles and haplotypes of the four markers spanning our linked region. The highest significance was obtained for a common haplotype of D10S197 and D10S193 (Transmitted/Untransmitted = 62/27, nominal $p = 0.0002$, not significant after correction for multiple tests). Because of the large number of alleles (33) and two-way haplotypes (689) examined, the actual significance of the results is not clear. Moreover, the interval is quite large for linkage disequilibrium, and, if the association is a real one the interval could be expected to contract with larger cohort sizes.

Discussion

We found evidence for linkage of an obesity phenotype, a BMI of 27 or more, with chromosome 10 markers. The results were consistent across cohorts of European-American and African-American ancestry. The convergence of evidence for linkage in our two independent cohorts as well as the previously reported linkage to a similar phenotype in French and German cohorts provides support for the presence of an obesity quantitative trait locus in 10p12.

A total of 44 different human genomic locations have been linked to obesity [1]. False positive findings for a complex trait such as obesity are likely to be common, and it is therefore crucial to distinguish real from false linkages. One of the ways to guard against false positive results is with very low p values [10]. A low p value can be found in the original report on the French cohort [2], but independent replication is still necessary to justify gene identification efforts. The confirmation of linkage to obesity in four separate cohorts should encourage molecular studies to find susceptibility gene(s).

It is important to keep in mind the nature of the phenotype linked to this region. The relatively low BMI of threshold 27 kg/m^2 or more suggests that even mild obesity could be influenced by the presumed genetic locus, provided the mild phenotype occurs in families identified by an extremly obese individual. This suggestion is reinforced by the observation that quantitative variation among family members was not linked to the region in our two cohorts and, at the least, was not described in the French and German cohorts. It is possible that the ascertainment has enriched the cohort for families with a high frequency of susceptibility alleles at the locus, much as would be expected in extreme selection under a polygenic model. If this model is correct, the next steps could be difficult.

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