Brief Genetics Report

The Quantitative Trait Locus on Chromosome 2 for Serum Leptin Levels Is Confirmed in African-Americans

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African-Americans experience higher rates of several chronic diseases, most notably diabetes, hypertension, coronary heart disease, and stroke, with a substantial proportion of the increased risk being attributable to obesity (1,2). Despite an increased prevalence of obesity among African-Americans and its significant impact on the health of this population, relatively few genetic studies have been conducted in this racial group. In two recently reported studies of Mexican-American and French families (5,15), a region of chromosome 2 containing the gene for pro-opiomelanocortin (*POMC*) was shown to exhibit strong evidence of linkage with levels of leptin, a plasma protein found to be highly correlated with an individual's total adiposity. Here we have attempted to replicate this finding in a large sample of African-Americans by typing a polymorphic marker (*D2S1788*) located ~15 cM from the *POMC* gene in a sample of 720 individuals in 230 families. In this sample of African-Americans, we detected significant linkage (logarithm of odds [LOD] = 1.26, $P = 0.008$) between *D2S1788* and leptin levels, with this quantitative trait locus (QTL) accounting for approximately half of the variation in leptin levels. This represents another replication of the results reported in Mexican-American and French families and confirms that a QTL in this region of chromosome 2 influences circulating leptin levels. Similar analysis was performed on BMI with an LOD score of 0.74 ($P = 0.03$), which meets the criteria of significance for a point hypothesis.

The recent isolation of the mouse *ob* gene and its human homologue (*LEP*) by positional cloning has provided a new opportunity to examine the genetic basis of obesity. Leptin, the protein product of *LEP*, is reliably assayed in plasma and is strongly correlated with an individual's total adiposity (3). We recently reported a substantial familial effect for leptin levels in African-Americans (4), suggesting a strong genetic component. Recently Comuzzie and colleagues were the first to report evidence of linkage (LOD = 4.95, $P = 9 \times 10^{-7}$) in a multipoint analysis of leptin levels in randomly ascertained pedigrees of Mexican-Americans with a QTL located on chromosome 2 very near the microsatellite *D2S1788* (5). Here, we report replication of linkage between this region of chromosome 2 and leptin levels in a sample of African-American families.

The families in this study were recruited from Maywood, Illinois, a predominantly minority working-class community adjacent to the western border of Chicago. This sample is part of a larger international collaborative study of environmental and genetic determinants of cardiovascular disease and associated risk conditions in contemporary populations of the African diaspora (6). The sample used in the present analysis consists of 720 African-Americans (268 men and 452 women) from 230 nuclear families. The mean age of the participants was 38.8 years, with a range from 11 to 85 years. Based on the sex-specific National Institutes of Health consensus panel criteria (7), the prevalence of overweight was 52% (men 35% and women 62%), and the prevalence of obesity was 29% (men 17.5% and women 35%). The study protocol was reviewed and approved by the Loyola University Medical Center Institutional Review Board.

Leptin concentration was determined by radioimmunoassay using the human leptin kit from Linco Research (St. Charles, MO), following the manufacturer's assay protocol. The intra-assay coefficient of variation for this assay was 3.2%. The microsatellite *D2S1788* (located ~72 cM from pter on chromosome 2) was typed using polymerase chain reaction with a fluorescently labeled sense oligonucleotide. Gels were scored using an automated DNA sequencer (Perkin Elmer, Foster City, CA) model 377 with Genescan 2.02 and Genotyper programs.

We used a variance component model applied to pedigree data to look for evidence of linkage between *D2S1788* and leptin levels in a sample of African-American families. This method is an extension of the strategy developed by Amos (8) to estimate the genetic variance attributable to the region around a specific genetic marker, as implemented in the analysis package SOLAR (9). This approach is based on specifying the expected genetic covariances between arbitrary relatives as a function of the identity-by-descent relationships at a given marker locus (9).

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LOD, logarithm of odds; QTL, quantitative trait locus.

TABLE 1
Maximum likelihood parameter estimates for serum leptin levels from the variance component linkage model comparing African-American and Mexican-American families

Parameters	Serum leptin	
	African-Americans	Mexican-Americans*
μ_{Men}	7.39 ± 1.09	14.60 ± 2.14
β_{Sex}	20.25 ± 1.33	18.94 ± 2.08
β_{Age}		
Men	0.12 ± 0.06	0.10 ± 0.06
Women	0.22 ± 0.04	0.02 ± 0.08
β_{Age^2}		
Men	0.003 ± 0.003	–0.004 ± 0.002
Women	–0.005 ± 0.002	–0.004 ± 0.002
SD	13.37 ± 0.39	17.16 ± 0.74
h^2	0.01 ± 0.24	0.24 ± 0.13
h^2_m	0.56 ± 0.21	0.47 ± 0.10
E^2	0.42 ± 0.08	0.30 ± 0.08

Data are maximum likelihood estimates ± SE. *Data taken from Comuzzie et al. (5).

Using the variance component model, we tested the null hypothesis that σ^2_{QTL}, the additive genetic variance due to the QTL, equals zero (no linkage) by comparing the likelihood of this restricted model with that of a model in which σ^2_{QTL} is estimated. A full explanation of this method can be found elsewhere (9). Since this analysis represents a test of a specific point hypothesis based on the detection of a significant linkage result from a previous study (5), we used a P value ≤ 0.05 as the criteria for establishing evidence of linkage replication.

The results of the present analysis, along with those of the Mexican-Americans, are displayed in Table 1. The mean serum leptin level from the Mexican-American men was about twice that of the African-American men. This is not surprising, however, since the Mexican-American men were considerably heavier. Interestingly, we were able to detect significant evidence of linkage (LOD = 1.26, P = 0.008) in this sample of African-American families between *D2S1788* and leptin levels, thereby replicating the earlier detection of linkage with a QTL in this same chromosomal region in Mexican-Americans (5). In the African-American sample, this locus accounted for 56 ± 21% of the variation in plasma leptin levels (h^2_{QTL}) (Table 1). This estimate of effect due to the QTL is consistent with that previously reported in Mexican-Americans (5). As a result, we not only replicate evidence of linkage between this region of chromosome 2 and plasma leptin levels, but we also show that the magnitude of the effect of this QTL is comparable across these two populations. This last point is of particular importance because it suggests that the effects of this QTL may be comparable across many populations, which would greatly increase its significance from a public health perspective.

Although the magnitude of the residual heritability (h^2) is different between the African-American and Mexican-American families (0.01 vs. 0.24, respectively) (Table 1), the 95% CI estimates (–0.23, 0.25) for the African-Americans include the residual heritability estimate of 0.24 obtained for the Mexican-Americans. These estimates are, therefore, not statistically different. However, the larger standard error obtained for the African-American families may be explained by the smaller pedigree size. Average pedigree size for the African-Americans was 3.1 (720 of 230), compared with 45.8 (458 of 10) for the Mexican-American families.

As previously reported (5), this region of chromosome 2 contains *POMC*, which is now considered a strong positional candidate gene for obesity (10). *POMC* codes for the prohormone pro-opiomelanocortin, which is post-transcriptionally processed to produce a number of hormones in the hypothalamic-pituitary axis, such as melanocyte-stimulating hormone and adrenocorticotropic hormone, which are suspected of being involved in obesity (11–13). In addition, a rare form of early-onset obesity has recently been attributed directly to a defect in *POMC* (14).

In summary, this finding represents a first replication of an earlier report of linkage of the microsatellite marker *D2S1788* with circulating leptin levels among Mexican-Americans and French families. Although this report does not isolate a particular susceptibility gene, it confirms the existence of a QTL determining serum leptin level and substantially strengthens the evidence for linkage with plasma leptin levels in the region of chromosome 2 containing *D2S1788*.

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