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A Combined Analysis of Genomewide Linkage Scans for Body Mass Index, from the National Heart, Lung, and Blood Institute Family Blood Pressure Program

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A combined analysis of genome scans for obesity was undertaken using the interim results from the National Heart, Lung, and Blood Institute Family Blood Pressure Program. In this research project, four multicenter networks of investigators conducted eight individual studies. Data were available on 6,849 individuals from four ethnic groups (white, black, Mexican American, and Asian). The sample represents the largest single collection of genomewide scan data that has been analyzed for obesity and provides a test of the reproducibility of linkage analysis for a complex phenotype. Body mass index (BMI) was used as the measure of adiposity. Genomewide linkage analyses were first performed separately in each of the eight ethnic groups in the four networks, through use of the variance-component method. Only one region in the analyses of the individual studies showed significant linkage with BMI: 3q22.1 (LOD 3.45, for the GENOA network black sample). Six additional regions were found with an associated LOD >2, including 3p24.1, 7p15.2, 7q22.3, 14q24.3, 16q12.2, and 17p11.2. Among these findings, the linkage at 7p15.2, 7q22.3, and 17p11.2 has been reported elsewhere. A modified Fisher's omnibus procedure was then used to combine the *P* values from each of the eight genome scans. A complimentary approach to the meta-analysis was undertaken, combining the average allele-sharing identity by descent ($\hat{\pi}$) for whites, blacks, and Mexican Americans. Using this approach, we found strong linkage evidence for a quantitative-trait locus at 3q27 (marker D3S2427; LOD 3.40, $P = .03$). The same location has been shown to be linked with obesity-related traits and diabetes in at least two other studies. These results (1) confirm the previously reported obesity-susceptibility locus on chromosomes 3, 7, and 17 and (2) demonstrate that combining samples from different studies can increase the power to detect common genes with a small-to-moderate effect, so long as the same gene has an effect in all samples considered.

Introduction

The prevalence of obesity has been increasing rapidly in the last several decades, especially in the industrialized countries. Obese individuals are at higher risk of morbidity and mortality from many chronic diseases, such as hypertension, non–insulin-dependent diabetes, lipid abnormalities, and coronary heart disease (Spadano et al. 1999). It is known that obesity-related traits, including BMI, are influenced by both genetic and environmental factors (Bouchard et al. 1998). In the majority of studies, heritability has been estimated to be 40%–90% (Borecki et al. 1998*b;* Bouchard et al. 1998; Barsh et al. 2000; Luke et al. 2001). Mutations responsible for several rare Mendelian types of obesity have been identified (Chagnon et al. 2000), although the genes that confer susceptibility to the common form of obesity are largely unknown. Segregation analyses have provided evidence of major genes influencing obesity-related traits (Price et al. 1990; Borecki et al. 1998*a*); however, other studies have inferred that multiple genes contribute, each having small effects individually (Bouchard et al. 1988; Hasstedt et al. 1997; Lecomte et al. 1997; Borecki 1998*b*). To search for the multiple unknown loci that are hypothesized, linkage analysis has been used with STR markers spread across the genome. Genomewide linkage analysis has been performed in several different populations, including people of European descent (Hager et al. 1998; Lee et al. 1999; Kissebah et al. 2000; Öhman et al. 2000; Van der Kallen et al. 2000; Watanabe et al. 2000; Perola et al. 2001), Mexican Americans (Comuzzie et al. 1997; Mitchell et

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al. 1999), Pima Indians (Hanson et al. 1998; Norman et al. 1998; Walder et al. 2000), and African Americans (Zhu et al. 2002).

Identifying the susceptibility genes for complex traits such as obesity, diabetes, and hypertension represents a challenging task for human geneticists. Multiple genes interacting with environmental factors usually affect these traits. Because of the moderate effect of each gene on the trait, it is difficult to acquire enough power to detect it with a moderate sample size. One potential solution to this challenge is to combine data from multiple studies (Li and Rao 1996; Gu et al. 1998; Lonjou et al. 1999; Palmer et al. 2001). In the present study, we first performed genome scans for BMI in the eight samples from the National Heart, Lung, and Blood Institute (NHLBI) Family Blood Pressure Program (FBPP). We subsequently conducted linkage analysis in the combined data set by means of a meta-analysis combining *P* values and also by pooling average allele-sharing identity by descent (IBD), $\hat{\pi}$.

Subjects and Methods

Family Ascertainment

Subjects were selected from the four component networks (GenNet, GENOA, HyperGEN, and SAPPHIRe) of the ongoing NHLBI FBPP. In brief, GenNet sampled black and white nuclear families through identification of young-to-middle aged probands with elevated blood pressure (BP). Both GENOA and HyperGEN sampled black and white sibships containing sib pairs with essential hypertension. GENOA also sampled Mexican American sibships containing sib pairs with hypertension, as well as some nonhypertensive sibs in all three ethnic groups. SAPPHIRe recruited three groups of Japanese and Chinese sib pairs: concordant for hypertension, concordant for hypotension, and extremely discordant. Anthropometric measurements were obtained at the time of the clinic visit. The pooled data set analyzed here currently includes genotype data and >120 measured and derived phenotypic variables in 6,849 participants, representing >60% of the total FBPP sample. In the present study, we used BMI as the measure of adiposity, because it is highly correlated with other measures of fat mass (Borecki et al. 1991) and is available for the largest number of individuals. Family relations were verified using the marker data; pedigree errors were corrected, and unrelated individuals were eliminated from the analysis.

Genotyping

DNA was extracted from whole blood by standard methods at each of the four networks and was sent to the Mammalian Genotyping Service in Marshfield, WI for genotyping (Center for Medical Genetics Web site). Screening Set 8 (372 highly polymorphic microsatellite markers) was used for all four networks. This screening set has an average heterozygosity of ~80% and an average intermarker distance of 10 cM and covers ~95% of the human genome.

Analytical Methods

The genome scans were performed separately for each of eight sampled populations—that is, for the GenNet white and black samples; the GENOA white, black, and Mexican American samples; the HyperGEN white and black samples, and the SAPPHIRe Asian sample—using the multipoint variance-component (VC) method implemented in the software GENEHUNTER 2 (Kruglyak et al. 1996; Kruglyak Lab Software Programs Web site). The VC method specifies the expected genetic covariances between relatives as a function of the estimated proportion of alleles shared IBD at a marker locus. The IBD probabilities were estimated using a multipoint approach that considers all available genotypes. The likelihood-ratio test was applied to test the null hypothesis of no additive genetic variance due to a quantitative-trait locus (QTL) at a particular location. Outliers for BMI were removed from each population before any analysis. Outliers were chosen as BMI observations that are not only far from the mean (e.g., >4 SD) but also separated from the nearest interior observation by $\geq$1 SD. BMI was transformed to approximate normality by taking the inverse normal transformation, since it is known that VC methods can be sensitive to departures from normality in the trait distribution (Allison et al. 1999). We also performed the genome scan using BMI without data transformation, and the results were quite similar (data not shown). Sex, age, and age squared were incorporated as covariates in the analysis. To evaluate significance of linkage, we performed simulations for groups with LOD >3.3, the proposed genomewide significant level (Pratt et al. 2000). We retained phenotypes and pedigree structure in the data sets and randomly generated genotypes under the hypothesis of no linkage. The simulations were repeated 100 times to obtain empiric *P* values for genomewide significance.

To combine the linkage evidence across the eight data sets, we used Fisher's omnibus procedure (Fisher 1932). This method is based on the observation that if n independent tests are made of the same hypothesis, resulting in P values $P_1, P_2, \ldots, P_n$, then we can calculate a combined P value for all n tests by $\Sigma_{i=1}^{n}(-2 \ln P_i)$, which is asymptotically distributed as a χ^2 distribution with $2n$ df. A modification of this method is needed to avoid bias in the pooling of nonparametric LOD scores from GENEHUNTER that are exactly zero (Province 2001). We used .0012 (LOD 2.0) as the suggested significance

level for the Fisher test statistic. Since linkage results may differ by ethnic group, we also produced a meta-analysis scan separately for whites and for blacks.

We further combined eight individual studies by pooling average allele-sharing IBD ($\hat{\pi}$) for each pair of individuals, estimated separately from each individual study, and performed genome scans using the original Haseman-Elston (HE) regression method (Haseman and Elston 1972) with the pooled $\hat{\pi}$. Because the frequency of individual alleles can vary substantially between different ethnic groups, and the estimation of IBD sharing is sensitive to allele frequencies in the absence of parental genotype data, we first calculated $\hat{\pi}$ for each pair of individuals from each individual study separately and then pooled $\hat{\pi}$ across the studies.

The probability of sharing i ($i = 0$, 1, or 2) alleles IBD ($\hat{f}_0$, $\hat{f}_1$, or $\hat{f}_2$, respectively) for each of the sib pairs was estimated using GENEHUNTER2 (the "dump ibd" subroutine) across the genome in each of the eight studies separately. The proportion of alleles shared IBD for each pair of relatives was calculated as $\hat{\pi} = \frac{1}{2}\hat{f}_1 + \hat{f}_2$. BMI was regressed against sex, age, and age squared within the study to obtain a residual suitable for analysis. This residual was considered as the phenotype in our linkage analysis, and we analyzed only sib pairs with nonmissing phenotype and genotype data. We then pooled samples by race over the different networks (whites and blacks). Because only one Mexican American population was available and because we had already combined the Chinese and Japanese samples for the individual genome scan, there was no pooling for Mexican Americans and Asians. Assuming that the variance of residuals was solely due to the genetic effect and a random environmental effect, we performed an HE regression using the model $y_i = \alpha + \beta\hat{\pi}_i + \varepsilon_i$, where y_i is the squared difference of the residuals of BMI adjusted for sex, age, and age squared for the ith sib pair, and $\hat{\pi}_i$ is an estimate of the marker locus IBD proportion. We further pooled whites, blacks, and Mexican Americans together. We did not include Asians in this final pooling exercise, because the mean and variance of the adjusted BMI were quite different from the other three groups and could add substantial heterogeneity to the analysis. To evaluate the significance of the combined IBD analysis, we permuted the age, age squared, and sex-adjusted phenotypes in the HE regression and recalculated the LOD scores through use of these permuted phenotypes. We did the permutation 100 times, to obtain the empiric genomewide P value.

Results

Family Characteristics

The clinical characteristics of the 6,849 family members are presented in table 1 for each study. The mean BMI values for whites, blacks, and Mexican Americans were similar but higher than that for Asians. The proportion of participants with a BMI >30 (a common definition of obesity) was much higher among whites, blacks, and Mexican Americans than among Asians. The lower average BMI in the subjects in the SAPPHIRe network is the result of their primary recruitment in Taiwan, where obesity is less common than in the United Sates. The heritability of BMI was calculated by use of the VC method and was 0.43–0.63 in the eight studies (table 1).

Single Genomewide Screen

The results of the genome screen in the eight studies considered separately are presented in figure 1. All of the regions with a LOD >2.0 from multipoint VC analysis are listed in table 2. If a nearby region has a LOD >1.0 in other populations, we also listed these regions in table 2 as "replicates." The analysis revealed one region with multipoint LOD scores >3.3, which is the approximate level of genomewide significance for this

Table 1

Characteristics of Family Members for BMI Genome Scan from the FBPP Study

Study and Population (*N*)	Age (years)	% Male	BMI	% BMI >30	Heritability
GenNet:					
White (606)	45.0 ± 14.1	47.2	29.2 ± 6.1	38.5	.59
Black (621)	40.5 ± 11.7	40.5	30.2 ± 8.3	42.3	.57
GENOA:					
White (753)	56.3 ± 9.8	47.3	30.0 ± 6.2	41.4	.43
Black (617)	56.5 ± 9.4	34.5	30.4 ± 6.3	45.5	.45
Mexican American (788)	55.8 ± 11.6	38.85	30.8 ± 6.1	50.4	.63
HyperGEN:					
White (1,138)	60.8 ± 9.4	47.31	30.4 ± 6.0	43.4	.56
Black (1,256)	50.7 ± 11.0	35.33	32.4 ± 7.6	56.9	.62
SAPPHIRe:					
Asian (1,070)	54.0 ± 12.2	43.6	25.6 ± 3.8	9.9	.57

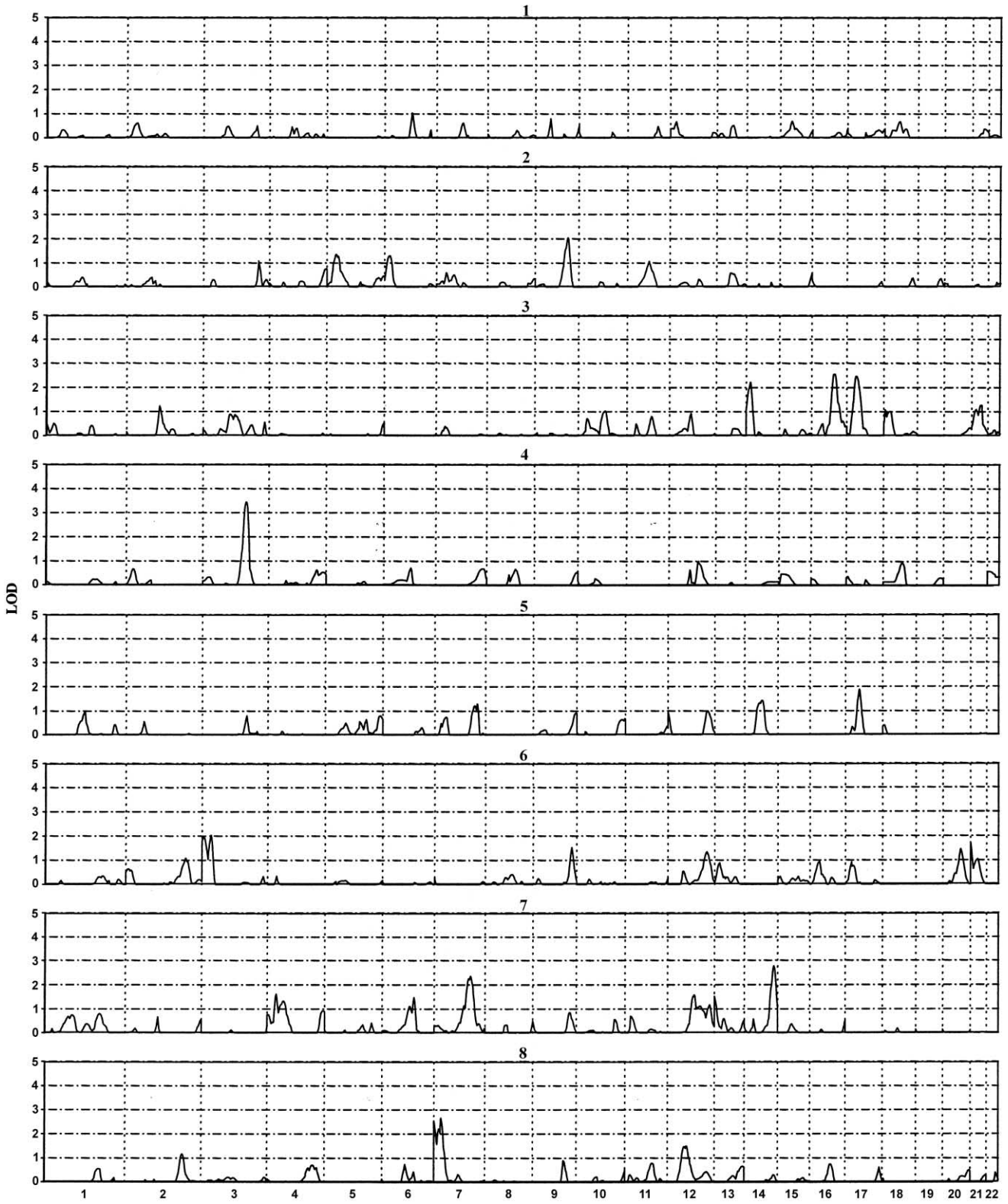

Figure 1 Multipoint VC LOD scores for linkage to BMI in each of the eight groups. 1 = GenNet white; 2 = GenNet black; 3 = GENOA white; 4 = GENOA black; 5 = GENOA Mexican American; 6 = HyperGEN white; 7 = HyperGEN black; and 8 = SAPPHIRe Asian. The *X*-axis is the chromosome location. Each chromosome is scaled according to its length from the genetic map and is separated from the others by vertical dotted lines.

Table 2

Results of Linkage Analysis from Eight Genome Scans

Chromosome and Marker	Location (cM)	Region (cM)	LOD Score	Study (Population)
3:				
D3S1259	31.17	0–37	2.03	HyperGEN (white)
D3S1764	145.45	138–155	3.45	GENOA (black)
7:				
D7S3051	24.89	0–32	2.66	SAPPHIRe (Asian)
D7S2847	124.39	108–137	2.36	HyperGEN (black)
D7S1824	146.62	124–151	1.29[a]	GENOA (Mexican American)
14:				
D14S617	100.71	88–112	2.15	HyperGen (black)
16:				
GATA67G11	78.71	66–88	2.55	GENOA (white)
17:				
D17S947	31.33	23–48	2.47	GENOA (white)
D17S2196	47.66	36–57	1.87[a]	GENOA (Mexican American)

[a] Replicate at the same region with LOD >2.

method of analysis at $\alpha = 0.05$ (Pratt et al. 2000). This region was centered at 3q22.1 (LOD 3.45 at marker D3S1764 in the GENOA black sample). In 100 simulated genomewide scans, we found a LOD >3.45 only once, which gave an empiric *P* value of .01. Six more regions were found with a LOD >2 (table 2) in at least one study. The regions are 3p24.1 (LOD 2.03, at marker D3S1259), 7p15.2 (LOD 2.66, at marker D7S3051), 7q22.3 (LOD 2.36, at marker D7S2847), 14q24.3 (LOD 2.15, at marker D14S617), 16q12.2 (LOD 2.55, at marker GATA67G11), and 17p11.2 (LOD 2.47, at marker D17S947).

Combined Analyses

The genome scan results pooling the studies with Fisher's method are presented in figure 2. We first combined the *P* values for the three white populations and the three black populations separately. Two regions showed suggestive linkage, with LOD >2, in whites:

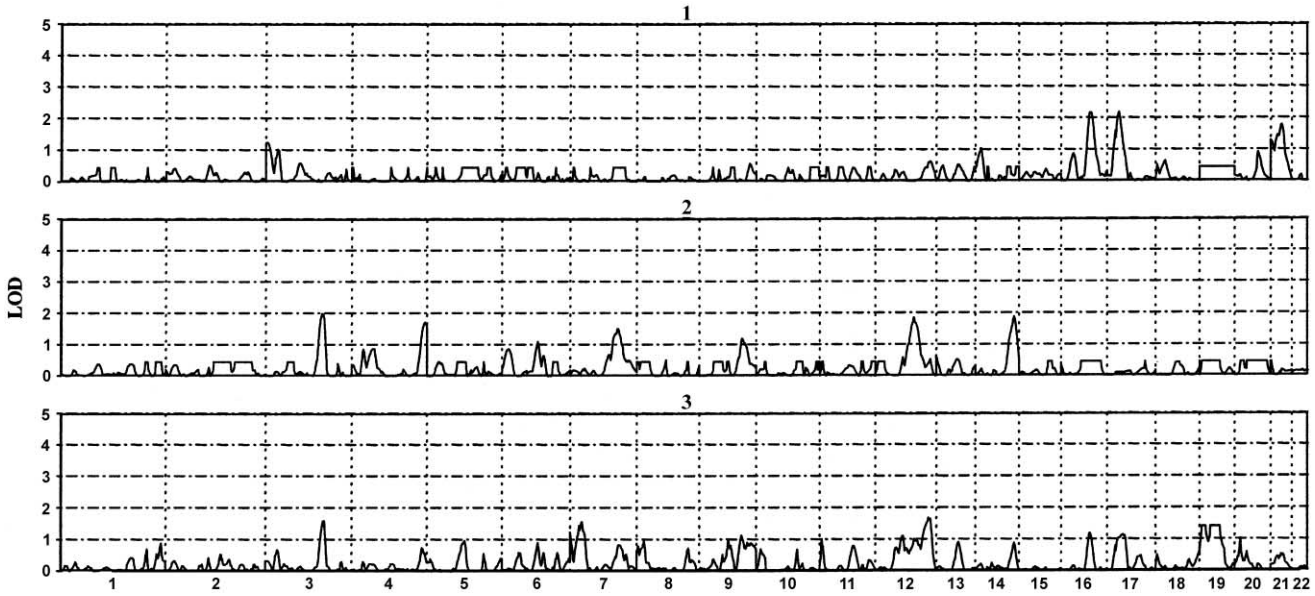

Figure 2 Results from combining *P* values by means of the modified Fisher's method. 1 = combined white; 2 = combined black; and 3 = all groups combined. Scaling and separation of each chromosome are as described in figure 1.

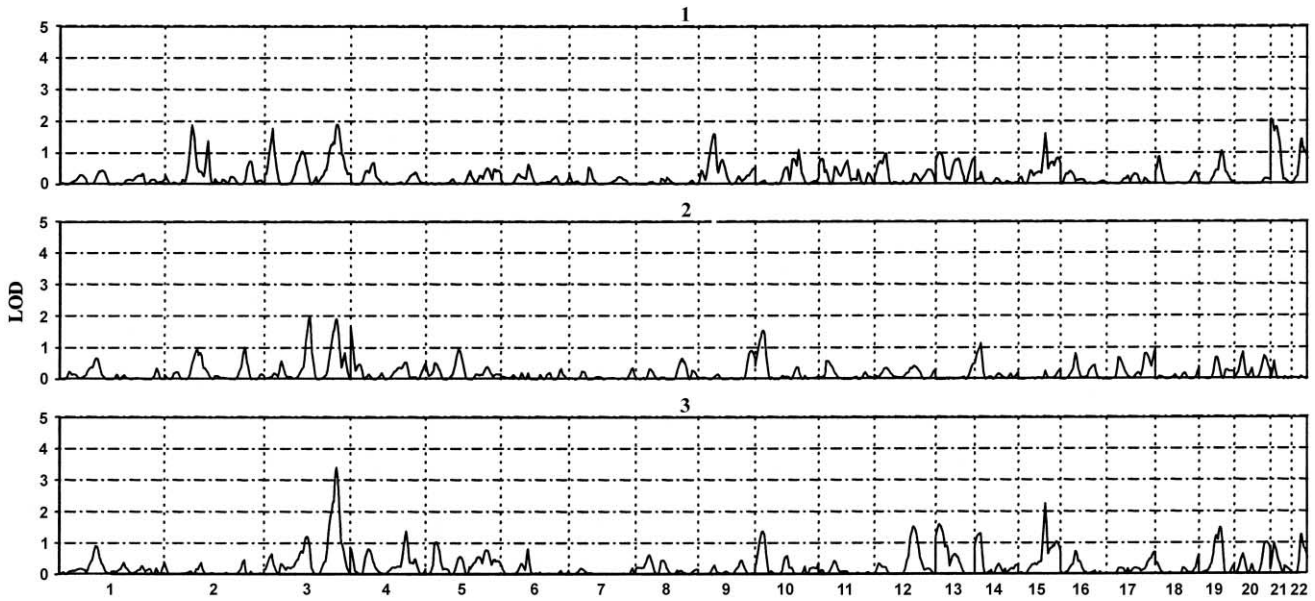

Figure 3 Results from combining IBD-sharing method. 1 = combined white; 2 = combined black; 3 = combined white, black, and Mexican American. Scaling and separation of each chromosome are as described in figure 1.

16q12.2 (LOD 2.15, at marker GATA67G11) and 17 (LOD 2.18, at marker D17S947). Next we combined *P* values across all eight studies; however, no region showed evidence of linkage with LOD >2. The highest LOD score is around marker D3S1764 (151.3 cM, with LOD 1.58). This is the only region showing strong linkage with BMI in individual studies.

The genome scan result for the combined IBD analysis is presented in figure 3. Table 3 shows the number of sib pairs selected in the analysis. Although no region showed evidence of linkage with LOD >2 in either the black or white samples, both show some evidence of linkage at the same location on chromosome 3 (186.97 cM, at marker D3S2427, with LOD 1.90 in whites vs. LOD 1.88 in blacks). When we combined white, black, and Mexican American populations, we obtained a LOD of 3.40 at the same region (186.97 cM, at marker D3S2427). In 100 permutated genome scans, we found a maximum multipoint LOD >3.40 only three times, which gave a genomewide empiric *P* value of .03.

Discussion

In the present study, strong linkage evidence was found for BMI on chromosome 3 (3q27, with LOD 3.40 at marker D3S2427; $P = .03$), through use of a combined IBD analysis. At least two other analyses support linkage to this region. In a genome scan involving 507 nuclear white families, Kissebah et al. (2000) reported that a QTL on chromosome 3 (3q27) was strongly linked to six traits representing the fundamental phenotypes of metabolic syndrome, including BMI and waist and hip circumferences. The 1-LOD interval for BMI (183–200 cM) from the Kissebah et al. (2000) study is very similar to our result (180–193 cM). Vionnet et al. (2000) also report significant linkage with diabetes or glucose intolerance at age <45 years at the same region in French whites. It is known that obesity is correlated with diabetes, and it is possible that the same variants have an effect on both obesity and diabetes. Several potential candidate genes have been identified around this region. Among these genes, the adiponectin gene (MIM 605441) (at 3q27) seems the most interesting. Adiponectin is the most abundant gene transcript specific to adipose tissue (Maeda et al. 1996). It encodes a secreted protein that circulates in serum of normal individuals. Although the precise function of the adiponectin in energy expenditure and fat par-

Table 3

Number of Sib Pairs Selected in the Combined IBD Analysis

Population and Study	No. of Sib Pairs
White:	
GenNet	384
GENOA	924
HyperGEN	1,055
Black:	
GenNet	297
GENOA	893
HyperGEN	800
Mexican-American:	
GENOA	468

titioning remains unclear, it is known that its circulating level is inversely correlated with BMI (Arita et al. 1999), and its mRNA level is suppressed in the adipose tissue of obese animals and humans (Hu et al. 1996). Kissebah further suggested that the gene for one of glucose transporters (GLUT2 [MIM 138160], at 3q26-q27) might be relevant, at least for the metabolic syndrome (Kissebah et al. 2000). Another candidate gene in this region is apolipoprotein D (Apo-D [MIM 164160]) (at 3q26). Apo-D is a component of high-density lipoprotein and is closely associated with the enzyme lecithin:cholesterol acyltransferase, an enzyme involved in lipoprotein metabolism. In an association study, Vijayaraghavan et al. (1994) found that a marker in this gene was more common in persons with obesity ($P = .006$).

The only significant linkage in a single genome scan was found at 3q22.1, with a LOD of 3.45 ($P = .01$) in GENOA blacks. However, this region is ~30 cM away from the 3q27 region mentioned above, and it is hard to tell whether the two QTLs are the same. Further replication will be required to establish linkage with this region.

Positive evidence for linkage and association with obesity has been reported elsewhere for the leptin gene (MIM 164160) on chromosome 7 (Clement et al. 1996; Duggirala et al. 1996; Reed et al. 1996; Lapsys et al. 1997; Roth et al. 1997). Although we did not show strong evidence for linkage in our pooled analysis, we did find some evidence of linkage in this region in the HyperGEN black (LOD 2.36, at marker D7S2847) and GENOA Mexican American (LOD 1.29, at marker D7S1824) samples.

Kissebah et al. (2000) recently published strong linkage evidence for leptin level, on chromosome 17 (17p11) (LOD 4.97, at marker D17S947; 31–45 cM). Two of our samples show some evidence of linkage for BMI in the same region—namely, the GENOA whites (LOD 2.47, at marker D17S947; 23–48 cM) and GENOA Mexican Americans (LOD 1.87, at marker D17S2196; 36–57 cM). The candidate genes in this region include solute carrier family 4 of the insulin-specific facilitated glucose transporter (GLUT4 [MIM 138190]) and the receptor protein known to bind to globular "heads" of the complement C1q (gC1qR).

Another interesting region emerged at 7p15.2, where "suggestive" evidence for linkage was seen in Asians (LOD 2.66, at marker D7S3051) (table 2). An obvious candidate in this region is neuropeptide Y (NPY [MIM 162640]). NPY is one of the two critical neurochemicals that have been shown to influence feeding behavior. Intracerebroventricular infusion of NPY into the brains of rats results in insatiable eating and weight gain (Morley 1987; Beck et al. 1992). Bray et al. (1999) showed evidence of linkage of NPY with body weight ($P = .020$) and a composite measure of body mass and size ($P = .048$) in a Mexican American population.

The genome scans for human complex traits published to date are mostly based on relatively small sample sizes, and it is almost certain that some true linkage will be missed in a single genome scan study in which each gene has a moderate effect. This has recently been shown in a simulation study by Hirschhorn et al. (2001). Thus, replication of some of these findings across multiple studies is critical to identify the true linkage. In obesity genome scan studies, we are beginning to see some of these replications, as was recently reviewed by Comuzzie et al. (2001); this development is particularly encouraging. Four regions (on chromosomes 3, 7, and 17) in the present study have been associated with significant linkage to obesity in publications before, which extends these positive developments.

An obvious step to increase power in linkage analysis of complex traits is to pool data across studies (Lander and Kruglyak 1995; Allison and Heo 1998; Palmer et al. 2001). In a recent review article, Altmuller et al. (2001) compared 31 whole-genome scans for different human complex diseases, with regard to design, methods, and results. They found that the most obvious differences influencing success in finding linkage across studies was sample size. Two methods were used in the present study to combine all eight studies from four networks: combining P value and combining $\hat{\pi}$. Fisher's method for combining P value is very general and easy to implement; however, highly significant P values from a single study can determine the significance of the Fisher test statistic (Province 2001). Guerra et al. (1999) showed that pooling raw data usually has greater power and results in fewer false-positive results than does combining P values. By combining the original IBD-sharing estimates from all eight studies, we detected a QTL for BMI on chromosome 3 with significant support, although the evidence was weak in any of the individual studies.

We did not perform ascertainment correction when we applied the VC method, even though the families in the FBPP study were ascertained through hypertension and it is known that there is correlation between hypertension and obesity. This was guided by several considerations. First, on the basis of the simulation study by de Andrade and Amos (2000), the power to detect a major locus and the mean likelihood-ratio test were similar whether the data were corrected for ascertainment or not, although there is some excess of false-positive findings when there is a large genetic background. Their finding is consistent with that of Allison et al. (1999). Second, it is not clear which kind of ascertainment would be the most efficient in our case. When an inefficient ascertainment correction is used, it can decrease power (Comuzzie and Williams 1999).

In summary, by combining the data from eight studies, we found strong evidence for a QTL influencing BMI, in FBPP data, on chromosome 3 (3q27). The same region has been shown to be linked to several traits related to adiposity and diabetes, with highly significant statistical support in at least two other studies. We also replicated the findings on chromosomes 3, 7, and 17 in some of the individual genome scans. Our study shows that by combining different data sets—and, hence, increasing the sample size—we can improve the power to detect genes affecting human complex traits with moderate heritability, such as BMI.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for genetic maps)

Kruglyak Lab Software Programs, http://www.fhcrc.org/labs/kruglyak/Downloads/ (for GENEHUNTER)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for adiponectin [MIM 605441], GLUT2 [MIM 138160], Apo-D [MIM 107740], leptin [MIM 164160], GLUT4 [MIM 138190], and NPY [MIM 162640])

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