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A Whole-Genome Scan for Obstructive Sleep Apnea and Obesity

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Obstructive sleep apnea (OSA) is a common, chronic, complex disease associated with serious cardiovascular and neuropsychological sequelae and with substantial social and economic costs. Along with male gender, obesity is the most characteristic feature of OSA in adults. To identify susceptibility loci for OSA, we undertook a 9-cM genome scan in 66 white pedigrees (n = 349 subjects) ascertained on the basis of either an affected individual with laboratory-confirmed OSA or a proband who was a neighborhood control individual. Multipoint variance-component linkage analysis was performed for the OSA-associated quantitative phenotypes apnea-hypopnea index (AHI) and body mass index (BMI). Candidate regions on chromosomes 1p (LOD score 1.39), 2p (LOD score 1.64), 12p (LOD score 1.43), and 19p (LOD score 1.40) gave the most evidence for linkage to AHI. BMI was also linked to multiple regions, most significantly to markers on chromosomes 2p (LOD score 3.08), 7p (LOD score 2.53), and 12p (LOD score 3.41). Extended modeling indicated that the evidence for linkage to AHI was effectively removed after adjustment for BMI, with the exception of the candidate regions on chromosomes 2p (adjusted LOD score 1.33) and 19p (adjusted LOD score 1.45). After adjustment for AHI, the primary linkages to BMI remained suggestive but were roughly halved. Our results suggest that there are both shared and unshared genetic factors underlying susceptibility to OSA and obesity and that the interrelationship of OSA and obesity in white individuals may be partially explained by a common causal pathway involving one or more genes regulating both AHI and BMI levels.

Introduction

Obstructive sleep apnea (OSA [MIM 107650]) is a common disorder characterized by recurrent episodes of apnea (no airflow) and hypopnea (partially obstructed airflow) that occur during sleep and is associated with oxygen desaturation, sleep fragmentation, and symptoms of disruptive snoring and daytime sleepiness. Diagnosis is straightforward and is generally validated by laboratory sleep studies (Flemons et al. 1999). The primary measure of OSA is the apnea-hypopnea index (AHI) (Redline et al. 1991). OSA is now recognized as a common clinical entity with serious cardiovascular and neuropsychological sequelae and substantial social and economic costs (Redline and Young 1993*a*). In the developed nations, sleep apnea is increasing in prevalence and has been estimated to affect 2% of middle-aged women and 4% of middle-aged men (Young et al. 1993), ⩾1% of preschool children (Ali et al. 1993; Guilleminault and Pelayo 1998), and ⩾11% of the elderly (Ancoli-Israel et al. 1991; Janssens et al. 2000). Annual costs related to the diagnosis and treatment of severe OSA in the United States are estimated to be ⩾$3 million, with much higher costs projected for treatment of the large number of individuals with mild-to-moderate disease (Redline and Young 1993*b*).

Clinical disease likely results from multiple interacting genetic and environmental factors (Redline et al. 1995), and OSA has been associated with a number of "intermediate" pathogenic pathways, including central obesity, craniofacial morphology, muscle and connective tissue factors, and control of ventilation (Redline and Young 1993*a*). Along with male gender, obesity (MIM 601665) is the most characteristic feature of OSA in adults and is most commonly measured as an elevated BMI (Strohl and Redline 1996). However, the nature of the causal pathways involved in the relationship between BMI and AHI is uncertain (Redline and Tishler 2000).

Both nontwin family and twin studies have suggested an important genetic component to snoring, sleeping patterns, and OSA (Ferini-Strambi et al. 1995; Redline et al. 1995; Heath et al. 1998; Linkowski 1999; Buxbaum et al. 2002). The molecular genetics of OSA has received little attention to date, and previous investigations have been limited to candidate-gene association

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studies (Redline and Tishler 2000; Kadotani et al. 2001).

To identify susceptibility loci for OSA, we conducted a genomewide scan in 66 pedigrees of European American origin, comprising 349 subjects. To maximize the informativity and power of our study, families were sampled from 1,349 white participants in the Cleveland Family Study, a genetic-epidemiological study of OSA (Redline et al. 1995). In these families, we have previously demonstrated increased risk of disease and elevated levels of OSA-associated pathophysiological traits among first-degree relatives of probands with OSA (Redline et al. 1995). Since the mode of inheritance of OSA is complex and AHI and BMI are highly correlated, we conducted a multipoint model-free linkage analysis of both AHI and BMI. A variance-component approach appropriate for extended pedigrees was employed that utilized all of the available phenotypic and genotypic information (Almasy and Blangero 1998). Given the close association of obesity and OSA, we performed linkage analysis of the AHI with and without BMI adjustment, and vice versa.

Subjects and Methods

Families

The data analyzed are from the Cleveland Family Study, a cohort that was assembled to study the genetic epidemiology of OSA and was followed longitudinally. This sample includes 179 white pedigrees; methods of recruitment and clinical evaluation have been described elsewhere (Redline et al. 1995). In brief, the ascertainment criteria for probands with OSA was laboratory-confirmed OSA (with either an AHI ⩾20 or symptoms considered severe enough to warrant therapy). During the first 5 years of the study, control families were also studied. Control families were identified by randomly selecting an individual from a list provided by the probands of three neighbors who had at least two family members residing in the metropolitan area (Redline et al. 1995). All available first-degree relatives and selected second-degree relatives (half-sibs, aunts, uncles, and grandparents) of the ascertained probands with OSA or control individuals were invited to participate. Second-degree relatives were studied if they lived in the same household as members of the nuclear family or if previously sampled members of the family had been found to include two or more relatives with OSA.

Families were subselected for a whole-genome scan on the basis of expected informativity. To enhance statistical power, we chose only sibships with members in the extremes of the sample's AHI distribution. Subjects were considered to be in the high AHI extreme if they (i) were overtly affected with OSA (lab-confirmed, requiring treatment) or had AHIs that exceeded community age-specific threshold levels for identifying OSA (AHI >5 for those aged <25 years, AHI >10 for those aged 25–65 years, and AHI >15 for those aged >65 years) or (ii) had AHIs that exceeded the sex- and age-adjusted 80th percentile. The lower extreme consisted of those below the 20th percentile. A sibship was chosen for genotyping if at least two siblings were in either of the extremes and if at least one was in the high extreme. A three-generation family was selected for genotyping if a grandparent was in the high extreme and one or more grandchildren were in either extreme. A total of 349 subjects in 66 pedigrees were studied in the genome-screen linkage analysis (mean pedigree size 5.3; range 4–14).

The subjects analyzed had undergone two assessments at an average interval of 4.05 years (SD 2.16 years). The phenotypic data analyzed in the whole-genome scan relates to the most recent survey.

Participants gave written informed consent and completed a protocol that included questionnaires, an in-home sleep study, anthropometry, and a blood sample. All protocols were approved by the institutional review boards of local hospitals.

Questionnaire

Each participant aged ⩾13 years completed a modified version of the SCOR Sleep and Health Questionnaire (Kump et al. 1994), as described elsewhere (Redline et al. 1995); parents completed the SCOR Questionnaire for subjects aged <13 years. Pack-years of cigarette smoking were calculated as the product of the average number of cigarettes smoked per day (divided by 20, to convert to packs) and the duration of smoking (in years).

In-Home Sleep Studies (Polysomnography)

The major outcome variable was the AHI, the number of respiratory events (either cessations [apneas] or discrete discernible reductions [hypopneas] in airflow or chest wall impedance lasting ⩾10 s associated with a ⩾2.5% fall in oxygen saturation) per hour of estimated sleep time. AHI was determined using in-home, overnight polysomnography with a portable monitor (Edentec Model 1 or 2), measuring nasal/oral thermistry, chest wall impedance, finger pulse oximetry, and heart rate, as described elsewhere (Redline et al. 1995). This method has been shown to yield estimates of the AHI that have high night-to-night reproducibility and that correlate well with values obtained using in-laboratory polysomnography (Redline et al. 1991). Probands who were under treatment with nasal continuous airway pressure (CPAP) ($n = 63$; 16.8%) were assessed when not wearing CPAP.

Anthropometry

Height and weight were measured in stocking feet on a flat surface, through use of a tape measure and level and a portable scale, respectively. BMI was defined as the weight/height^2 (in kg/m^2).

Genotyping and Data Management

All subjects ($n = 349$) were genotyped by the National Heart, Lung, and Blood Institute Mammalian Genotyping Service (Center for Medical Genetics), using DNA that was extracted from whole blood or buccal scraping samples with Puregene Kits (Gentra Systems). We analyzed 375 autosomal STR markers, with a sex-averaged mean spacing of 9.1 cM, derived from Weber Marker Set 10. Marker and gene locations were determined using the Human Genome Working Draft (UCSC Genome Bioinformatics Web site) and SNPper (University of Arizona/Channing Labs Web site). The Kosambi mapping function was used for all multipoint analyses.

Possible errors in our pedigree and genotype data were assessed using the RELCHECK (Broman and Weber 1998), PEDCHECK (O'Connell and Weeks 1998), and MERLIN (Abecasis et al. 2002) programs. The mean rate of pedigree inconsistencies was less than one inconsistency per marker (0.75). Marker-allele frequencies were estimated, using the SOLAR program, by maximum-likelihood estimation (Almasy and Blangero 1998).

Statistical Analysis

The quantitative phenotypes included in the linkage analysis were AHI and BMI. AHI was skewed with a long right-hand tail and was therefore $\log_e$ transformed prior to analysis. Formal tests of model fit using the FISHER program (Lange et al. 1988) suggested that the ln (AHI) and BMI were acceptably close to multivariate normality. Covariate adjustments were performed within the linkage analysis, as described below. Modeling included the investigation of polynomial and interaction terms among covariates.

Two-point and multipoint linkage analysis of the genome-scan data was performed using a variance-component method, as implemented in the SOLAR program (version 1.7.4 running under Linux Mandrake version 8.0) (Almasy and Blangero 1998). SOLAR partitions the observed phenotypic variance into additive genetic and nongenetic components, by maximum-likelihood methods. Each model assumed that the distribution of the response phenotype in a pedigree was multivariate normal, with a mean that depended upon a particular set of explanatory covariates. Genotypes were imputed for untyped individuals, conditional on all other marker data and pedigree structure; and marker-specific identical-by-descent (IBD) matrices among all relative pairs were estimated independently for all autosomal markers. Multipoint IBD matrices were then generated at 1-cM resolution. Expected genetic covariances among relatives were specified as a function of the fixed effects of covariates, residual error, and random effects reflecting polygenic factors and an unobserved quantitative-trait locus (QTL) linked to an observed marker locus. The narrow-sense heritability (h_N^2) was defined as the ratio of variance due to additive genetic effects (σ_A^2) to the total phenotypic variance of each trait (Khoury et al. 1993): $h_N^2 = \sigma_A^2/\sigma_{Total}^2$.

Covariates potentially modeled in the variance-component analyses included age, age^2, sex, age*sex, history of surgery that may modify upper-airway patency (uvulopalatopharyngoplasty, tonsillectomy, or nasal septal surgery [$n = 138$; 39.5%]), self-reported alcohol consumption, pack-years, pack-years^2, height, and height^2. In certain analyses, ln (AHI) or BMI were modeled as covariates. Covariates that were significant at $P < .05$ were retained in the models.

The statistical associations between covariates entered as fixed effects and the response variable were assessed by removal of terms from the mean model and calculation of a likelihood-ratio χ^2 test statistic. The same approach was used as an approximate guide to the "significance" of a departure of the value of a variance component from its null value (zero).

The null hypothesis of no linkage at a specific chromosomal location was tested by comparison of a polygenic model to a model with genetic variance components for both a QTL and polygenic factors. Twice the difference in $\log_e$ likelihood of these two models gives a test statistic that is asymptotically distributed as a 1/2: 1/2 mixture of a χ_1^2 and a point mass at zero (Self and Liang 1987). The difference between these two $\log_{10}$ likelihood values corresponds to a LOD score for linkage.

The single ascertainment scheme was corrected for in the SOLAR program by conditioning the pedigree likelihood on the probability of the proband's phenotype for each outcome (Lange et al. 1988).

Simulations were performed using SOLAR, to assess the statistical significance of the linkage results. Genotypes for a fully informative unlinked marker were created, and the evidence for linkage was assessed in 100,000 replicates. The number of times that a LOD score exceeded a specified threshold provides an empirical P value for that LOD score threshold.

Results

Demographics in Families with OSA

A total of 349 individuals were included in the genome-scan data set. The characteristics of the study population included in the genome-scan linkage analyses are

presented in table 1. The extreme values for mean AHI and BMI in the probands are evident, as is the predominance of men and ever-smokers (table 1). Consistent with previous reports that the risk of developing OSA increases with age in whites (Stradling 1995), parents had higher mean AHI values than siblings, and siblings had higher mean AHI values than children of probands. Males and females were equally represented in the study population as a whole (table 1). Among probands with laboratory-diagnosed OSA, no significant differences in mean ln (AHI) or BMI were observed in the group that had received upper-airway surgery versus other forms of treatment (surgery: mean ± SEM ln (AHI) = 3.78 ± 0.11, BMI = 34.82 ± 1.47; nonsurgery: ln (AHI) = 3.64 ± 0.17, BMI = 38.24 ± 2.00).

Phenotypic Modeling

Variance-component analyses using Fisher and SOLAR suggested that, after adjustment for all covariates, the h^2_N of the ln (AHI) levels was 36.3% (SE 9.9%)—that is, additive genetic effects (σ^2_A) contributed just over one-third of the total variance. σ^2_A was significantly greater than zero ($P < .0001$). The variance-component modeling suggested that sex ($P < .001$) and linear ($P < .0000001$) and quadratic ($P = .03$) terms for age were highly significant for ln (AHI). Levels were higher in older subjects and in males. None of the other potential covariates, including surgical treatment, were significantly associated with the ln (AHI) level.

The h^2_N of BMI was 52.8% (SE 8.8%); σ^2_A was significantly greater than zero ($P < .0000001$). Linear ($P = .0004$) and quadratic ($P < .0000001$) terms for age were highly significant for BMI; levels were higher in older subjects. None of the other potential covariates, including sex and surgical treatment, were significantly associated with BMI.

Ln(AHI) and BMI were closely associated with each other ($P < .0000001$). The h^2_N of the ln (AHI) levels after inclusion of BMI as an additional covariate was reduced to 32.8% (SE 11.1%); the h^2_N of BMI after inclusion of ln (AHI) as an additional covariate was reduced to 42.9% (SE 10.6%). These associations were independent of age and sex, and the covariance model ensured appropriate adjustment for familial correlation.

Genomewide Multipoint Variance-Component Linkage Analysis

Genomewide multipoint variance-component linkage results obtained using SOLAR for the ln (AHI) and BMI are presented in figures 1 and 2 and are summarized in table 2. For ln (AHI), 12 multipoint LOD scores >1.0 were found on 13 chromosomes. The highest LOD scores were 1.64 on chromosome 2 (74 cM from pter, at marker D2S1352; 2p16), 1.43 on chromosome 12 (7 cM, flanked by markers D12S372 and GATA49D12; 12p13), and 1.40 on chromosome 19 (74 cM, flanked by markers D19S245 and D19S559; 19q13) (table 2). After adjustment for BMI, 10 of these linkages were greatly reduced (LOD score <0.7), suggesting that any susceptibility loci in these regions modulating AHI act through a pathway also involving BMI. However, the linkages on chromosomes 2p and 19q remained essentially unchanged, suggesting that these potential linkages were to gene(s) modulating AHI largely independently of BMI (table 2).

For BMI, 15 multipoint LOD scores >1.0 were found on 12 chromosomes. The highest LOD scores were 3.41 on chromosome 12q (89 cM from pter, flanked by markers D12S1052 and D12S1064; 12q21), 3.08 on chromosome 2p (56 cM, at marker D2S1788; 2p22), and 2.53 on chromosome 7p (5 cM, flanked by markers D7S3056 and D7S2477; 7p22) (table 2). After adjustment for ln (AHI), 11 of these linkages were greatly reduced (LOD < 1.0), suggesting that any susceptibility loci in these regions modulating BMI may also act through a pathway involving AHI (table 2). The linkages to BMI on chromosomes 2p, 7p, and 12q were roughly halved after inclusion of ln (AHI) as a covariate but re-

Table 1

Characteristics of the Famlies Studied in the OSA Genome Scan

	Mean (SD)						% (No.)			
Sample	Age (years)	AHI[a]	Height (cm)	Weight (kg)	BMI (kg/m^2)	Pack-Years of Smoking	Male	Current Smoker	Former Smoker	Nonsmoker
Probands (n = 63)[b]	53.0 (11.6)	38.6 (31.9–46.6)	174.6 (8.3)	108.5 (27.2)	35.6 (9.1)	22.9 (34.4)	87.3 (55)	14.3 (9)	50.8 (32)	34.9 (22)
Parents (n = 31)	72.6 (9.2)	25.2 (19.4–32.9)	164.2 (9.6)	81.9 (17.8)	30.3 (6.0)	18.5 (25.9)	45.2 (14)	9.7 (3)	35.5 (11)	54.8 (17)
Children (n = 89)	24.8 (10.9)	5.6 (4.7–6.6)	166.8 (12.6)	79.1 (23.8)	28.0 (6.8)	1.7 (5.0)	49.4 (44)	14.6 (13)	12.4 (11)	73.0 (65)
Siblings (n = 84)	47.2 (13.1)	12.9 (10.5–15.8)	169.1 (9.0)	91.8 (24.6)	32.2 (9.6)	10.1 (15.5)	46.4 (39)	20.3 (17)	32.2 (27)	46.5 (39)
Other relatives (n = 54)	31.8 (21.8)	6.9 (5.2–9.0)	164.2 (12.9)	77.4 (32.0)	27.9 (9.1)	6.1 (12.7)	48.2 (26)	20.4 (11)	14.8 (8)	64.8 (35)
Spouses (n = 28)	53.6 (9.9)	7.2 (5.6–9.5)	164.6 (7.2)	83.5 (19.0)	30.9 (7.3)	5.1 (10.4)	14.3 (4)	7.1 (2)	32.1 (9)	60.7 (17)

[a] Data are geometric mean (95% CI).

[b] Probands with OSA. Three pedigrees studied were ascertained on the basis of probands who were community control individuals; data on these three probands were included in the "other relatives" category of this table.

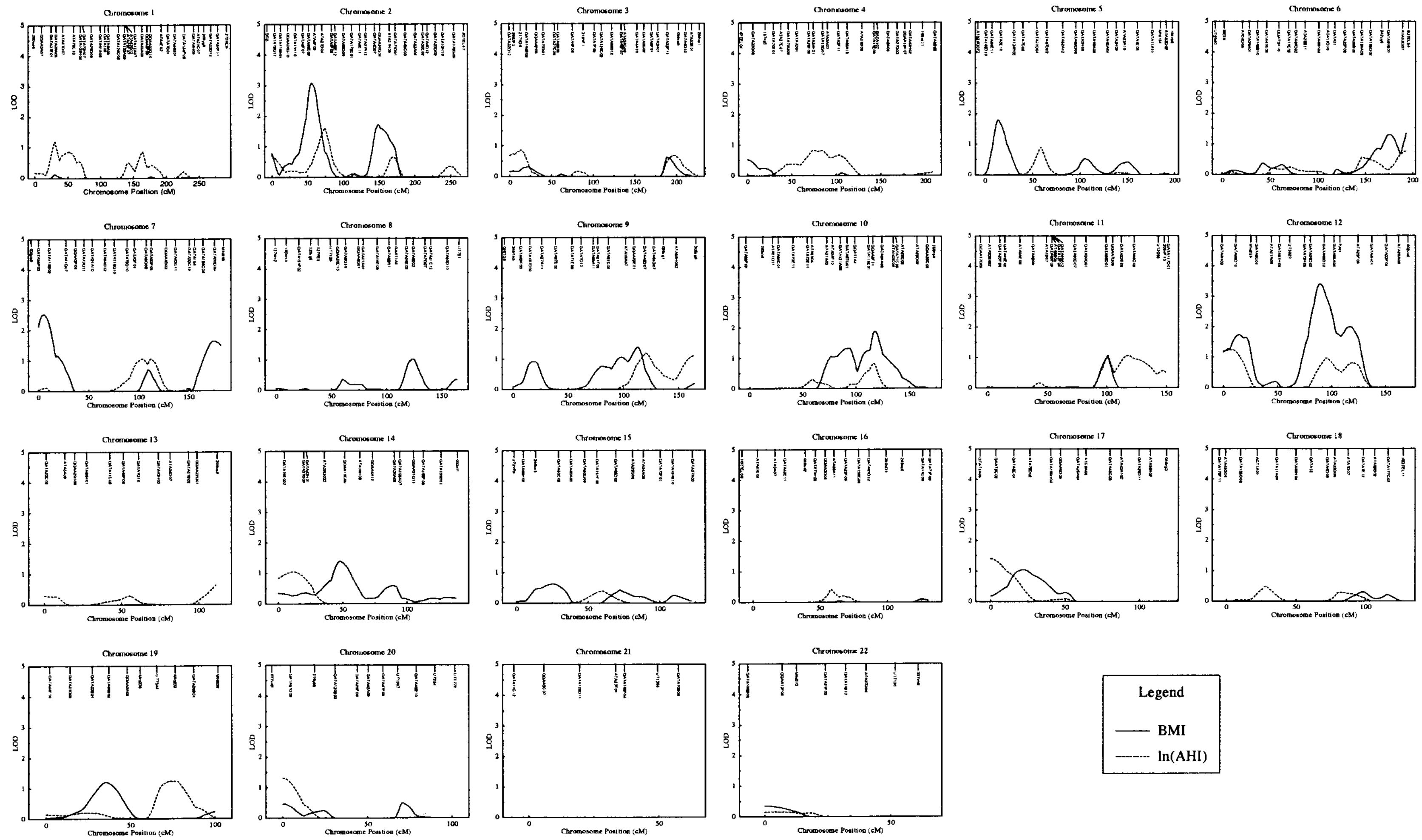

Figure 1 Multipoint variance-component linkage analysis of all 22 autosomes in pedigrees with OSA. Linkage analysis results are presented for ln (AHI) and BMI, with adjustment for relevant covariates. The *X*-axis represents genetic distance (in cM) along each of the 22 autosomes, and the *Y*-axis represents the multipoint variance-component LOD score. Markers are arrayed in map order along top of each plot.

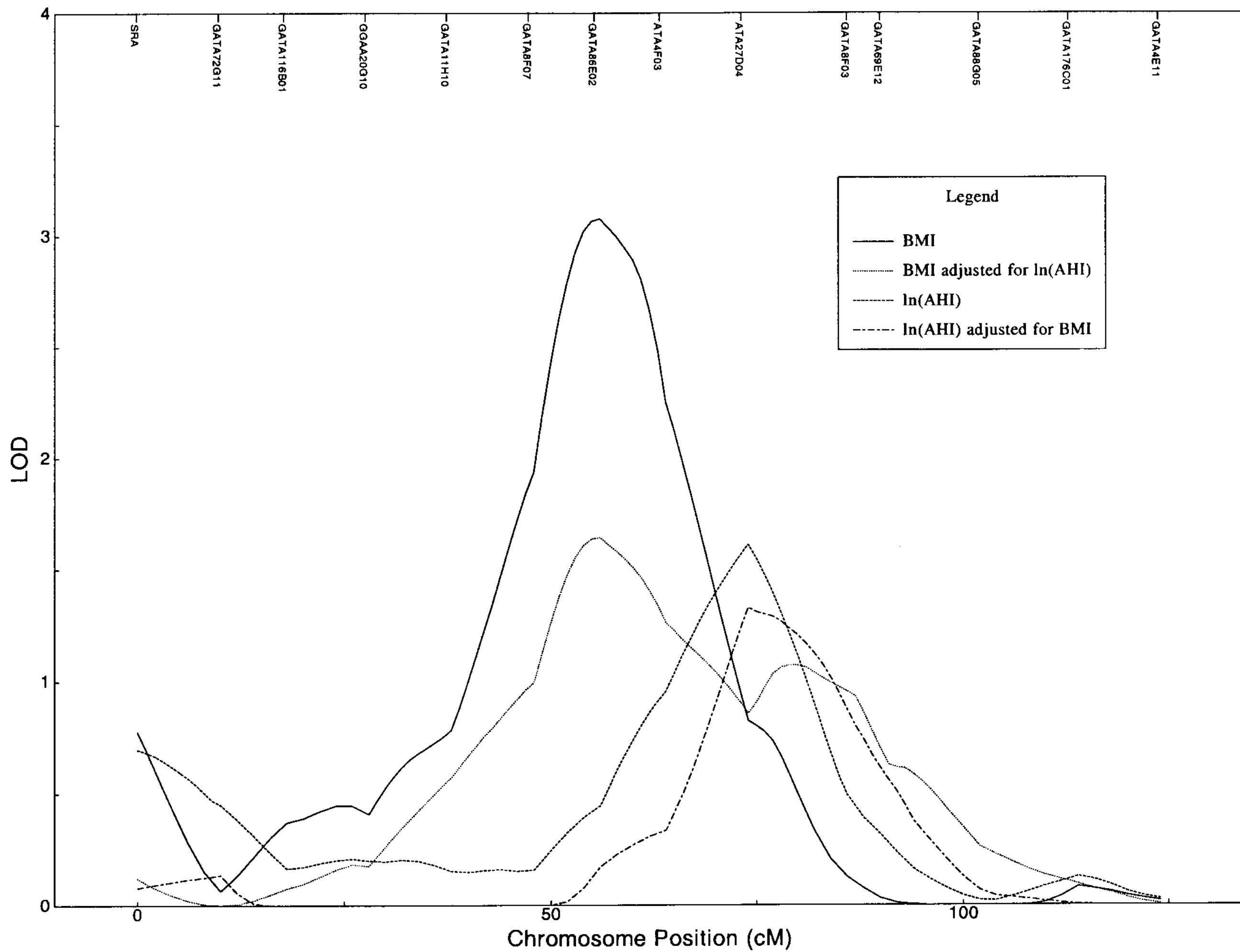

Figure 2 Multipoint variance-component linkage analysis of chromosome 2p (0–125 cM from pter). Genetic distance (in cM) is plotted against the multipoint variance-component LOD score for ln (AHI), ln (AHI) adjusted for BMI, BMI, and BMI adjusted for ln (AHI). Chromosome 2 markers are arrayed in map order along the top of the plot.

mained >1 (table 2), suggesting that any susceptibility genes in these regions may have both direct and indirect (via a pathway involving AHI) effects on BMI.

Several broad chromosomal regions demonstrated some evidence for linkage to both ln (AHI) and BMI. The chromosomal region that demonstrated the most evidence for linkage to ln (AHI) on chromosome 2p (LOD score 1.64) was also associated with strong evidence for linkage to BMI (LOD score 3.08) (fig. 2). Similarly, the chromosome 6, 9, and 11 regions that gave some evidence of linkage to ln (AHI) also gave some evidence of linkage to BMI (table 2).

To estimate the statistical significance of the ln (AHI) and BMI linkage results, we performed simulations with a fully informative unlinked marker, followed by variance-component linkage analysis of each phenotype for each replicate. The highest observed two-point LOD scores of particular interest in the ln (AHI) linkage analysis were LOD = 1.72 for D2S1352 (chromosome 2 at 74 cM) and LOD = 1.22 for D19S246 (chromosome 19 at 78 cM). In 100,000 replicates of an unlinked marker, LOD scores ⩾1.72 were observed 244 times; thus, the empirical P value for the D2S1352 linkage to ln (AHI) was .002. Similarly, the empirical P value for the D19S246 linkage to ln (AHI) was .009. The highest observed two-point LOD scores in the BMI linkage analysis were LOD = 2.68 for D12S1064 (chromosome 12 at 95 cM; empirical $P = .0002$) and LOD = 2.42 at D2S1788 (chromosome 2 at 56 cM; empirical $P = .0004$). Although the simulations were based on two-point linkage analysis, it is of interest that the multipoint LOD score for BMI on chromosome 2p (3.08) was exceeded in only 8 of 100,000 simulations ($P = .00008$).

Table 2

Genome Scan Multipoint Linkage Analysis with SOLAR: Chromosomal Regions with LOD Scores >1.0

	Linkage with ln (AHI)			Linkage with BMI		
		Maximum Multipoint LOD Score			Maximum Multipoint LOD Score	
Chromosome	Position (cM)	ln (AHI)[a]	ln (AHI) Adjusted for BMI[b]	Position (cM)	BMI[c]	BMI Adjusted for ln (AHI)[d]
1	30	1.39	.32	…	…	…
2	74	1.64	1.33	56	3.08	1.64
2	…	…	…	149	1.72	.28
5	59	1.07	.47	14	1.81	1.08
6	190	1.16	.002	193	1.33	.02
7	112	1.29	.01	5	2.53	1.07
7	…	…	…	175	1.65	.71
8	…	…	…	124	1.02	.32
9	120	1.25	.51	113	1.39	.80
10	…	…	…	118	1.87	.60
11	101	1.07	.47	101	1.03	.51
12	7	1.43	.37	14	1.73	.19
12	…	…	…	89	3.41	1.84
14	12	1.17	.05	48	1.39	.83
17	1	1.11	.24	22	1.04	.11
19	74	1.40	1.44	36	1.21	.20
20	0	1.31	.22	…	…	…

[a] ln (AHI) adjusted for age, age^2, and sex.
[b] ln (AHI) adjusted for age, age^2, sex, and BMI.
[c] BMI adjusted for age and age^2.
[d] BMI adjusted for age, age^2, and ln (AHI).

Discussion

We have conducted a whole-genome scan for OSA through use of a sample of pedigrees selected to maximize likely informativity for linkage. Furthermore, in recognition of the strong and consistent epidemiological and clinical associations between obesity and OSA, we have explored the interrelationship between the linkages to AHI and BMI. Our genomewide linkage scan is the first reported investigation of linkage in pedigrees with OSA and has found evidence of linkage to several chromosomal regions.

The importance of familial determinants in the regulation of BMI in humans is well established. Studies of pedigrees and twins have suggested that BMI has a heritability of 50%–80% (Stunkard et al. 1986; Allison et al. 1996; Maes et al. 1997), consistent with our results. The heritability of AHI levels has not previously received much attention, and it is of interest that our results show strong genetic effects underlying this trait. The majority of the observed familial correlations in BMI and AHI were attributable to genetic rather than environmental factors. Previous genetic studies of obesity have not evaluated OSA, which may occur in as many as 66% of obese individuals. Although obesity is a risk factor for OSA, it is also plausible that OSA may increase risk for obesity. For example, OSA causes sleep fragmentation and sleepiness, effects that may promote weight gain via reduced physical activity and hypercytokinemia (Vgontzas et al. 2000). The results of the phenotypic modeling were consistent with the hypothesis that obesity and OSA have both shared and unshared genetic determinants. Specifically, our study shows that the familial correlations in BMI are reduced but remain significant after adjustment for the AHI. Similarly, the familial correlations in AHI are reduced but remain significant after adjustment for the BMI.

The highest observed multipoint LOD score for ln (AHI) was 1.64, on chromosome 2p16. This result is not significant at a genomewide level but is suggestive evidence of linkage in our data set (Lander and Kruglyak 1995). The 2p region also gave promising evidence for linkage to BMI (LOD score 3.08; 2p22), corresponding to significant linkage in our data set. The chromosome 2p linkages may represent a pleiotropic locus affecting both AHI and BMI independently, with additional effects on BMI modulated through the effects on AHI (fig. 2). Alternatively, the linkages to AHI and BMI on chromosome 2p may represent two or more distinct loci. Suggestive linkage to ln (AHI) was found on chromosome 19q13. The chromosome 19q linkage may represent the action of a locus affecting AHI independently of BMI—that is, a genetic pathway distinct from that involved in the determination of body mass.

Multiple genomic regions have been linked to obesity-related phenotypes in genome-scan linkage studies (Comuzzie and Allison 1998); as of October 2001, 59 loci had been linked to obesity measures in genomewide scans of humans (Rankinen et al. 2002). Evidence for linkage of obesity-related phenotypes to the three most promising regions for BMI in the present study, chromosomes 2p, 7p, and 12q, has been reported in previous studies. The chromosome 2p region has been linked to BMI, fat mass, and skinfold thickness in previous linkage studies (Wilson et al. 1991; Bailey-Wilson et al. 1993; Comuzzie et al. 1997; Rotimi et al. 1999; Delplanque et al. 2000). The 7p (Bray et al. 1999; Chagnon et al. 2000) and 12q (Sun et al. 1999; Perusse et al. 2001) regions have also been previously linked to obesity measures.

Several biologically plausible candidate genes are located within the most promising chromosomal regions in our analysis (candidate-gene locations were obtained from the June 2002 freeze at the UCSC Genome Bioinformatics Web site). The chromosome 2p region contains acid phosphatase 1 (MIM 171500), apolipoprotein B precursor (MIM 107730), proopiomelanocortin (MIM 176830), and the alpha-2B-adrenergic receptor (MIM 104260), all of which are biologically plausible candidates for obesity and have been previously associated with BMI, percentage of body fat, and/or serum leptin levels (Rajput-Williams et al. 1988; Lucarini et al. 1990; Saha et al. 1993; Pouliot et al. 1994; Lucarini et al. 1997; Krude et al. 1998; Heinonen et al. 1999; Hixson et al. 1999; Miraglia del Giudice et al. 2001). The 7p region contains the neuropeptide Y (MIM 162640) gene, which has been associated with BMI and waist:hip ratio (Bray et al. 2000). The 12q region contains the vitamin D receptor (MIM 601769) and insulin-like growth factor 1 (MIM 147440) genes, which have been associated with BMI and percentage body fat (Sun et al. 1999; Ye et al. 2001). The chromosome 19q region contains the apolipoprotein E (MIM 107741) gene, which regulates lipid metabolism and has been associated with serum leptin levels, measures of obesity, and elevated AHI (Kadotani et al. 2001; Oh and Barrett-Connor 2001; Yanagisawa et al. 2001).

Obesity appears to increase risk of OSA ~10–14-fold, with the most marked effects observed in middle-aged subjects (Guilleminault et al. 1976; Strohl and Redline 1996; Redline et al. 1998). Furthermore, weight loss, even of modest amounts, may reduce the severity of OSA (Smith et al. 1985). Obesity may increase susceptibility to OSA through fat deposition in upper-airway tissues, reducing nasopharyngeal caliber, and/or from hypoventilation occurring in association with reduced chest wall compliance. It is also possible that the association between obesity and OSA may be partly based on pleiotropic effects, as may occur if the same gene or set of genes influences ponderosity and ventilatory control and/or craniofacial morphology. The co-occurrence of OSA, central obesity, hypertension, and type 2 diabetes suggests that OSA may be part of a "metabolic" syndrome (Catterrall et al. 1984; Katsumada et al. 1991; Strohl et al. 1994; Strohl 1996; Ficker et al. 1998; Elmasry et al. 2000, 2001; Al-Delaimy et al. 2002), which may be largely influenced by genes that influence insulin resistance and body fat distribution (McCarthy 2002). Candidate genes for obesity are therefore relevant for studies of the genetics of OSA, both because of the prominence of obesity in the OSA phenotype and because of the potential impact that these genes have on the expression of other traits of potential relevance to OSA.

There is growing evidence that leptin, an adipose-derived circulating hormone, may have such pleiotropic effects (Boston 2001). In addition to the role of leptin in appetite regulation and energy expenditure, mouse models suggest that it also influences lung growth (Tsuchiya et al. 1999) and respiratory control (O'Donnell et al. 1999). Studies after leptin replacement in mutant obese mice (Lep^{ob}) suggest that leptin deficiency causes depressed ventilatory responses to hypercapnia in both wakefulness and sleep (O'Donnell et al. 1999). Although leptin-deficient mouse models have not been studied for OSA per se, these studies provide important supporting data implicating the pleiotropic effects that a single protein has on several aspects of the OSA phenotype (obesity, abnormal ventilatory control, and disturbed sleep architecture) (O'Donnell et al. 1999; Sinton et al. 1999; Tsuchiya et al. 1999). Of great interest in this context, the same marker giving maximal evidence of linkage to BMI in the 2p region in our study (D2S1788) has also shown evidence of significant linkage to leptin levels in multiple studies (maximum LOD score 2.8–7.5) (Comuzzie et al. 1997; Hixson et al. 1999; Rotimi et al. 1999), as have nearby markers (Hager et al. 1998; Delplanque et al. 2000). This region on chromosome 2 encompasses the gene encoding POMC, which may be important in the regulation of appetite, obesity, and variation in leptin levels (Hixson et al. 1999; Miraglia del Giudice et al. 2001). Given that we found linkage to both AHI and BMI on chromosome 2p, POMC is a biologically plausible candidate that could explain the observed results and would be consistent with a genetically regulated metabolic component to OSA pathogenesis.

In general, we observed stronger evidence of linkage for BMI than AHI. We hypothesize that this is not due to a larger contribution of environmental influences on AHI than BMI, since most known environmental influences on OSA have relatively modest effects (Redline and Young 1993*a*). Rather, it is likely that BMI is assessed with less measurement error and greater day-to-day consistency than is AHI, which is derived from an

overnight recording and processing of multiple channels of physiological data (Redline et al. 1991). Future genetic studies of OSA may benefit from ongoing advances in the physiological processing of polysomnographic data.

The present study has several potential limitations. Our sample, although intensively phenotyped, was of moderate size. Our limited power to detect modest effects may at least partially explain the modest linkages we found to AHI. The generalizability of linkage results in the families with OSA that we studied to OSA in other populations with different exposures is undetermined. There are well-described ethnic differences in upper-airway anatomy that may alter the expression of the genetic determinants of OSA among other ethnic groups (Redline et al. 1997). We have included potential environmental determinants of OSA, such as cigarette smoking and alcohol consumption, as covariates in our analyses, but we have not formally tested for genotype-by-environment interactions. Although the quantitative trait that we analyzed (AHI) is the most common metric used to identify and quantify OSA, it is possible that more sophisticated indices of overnight breathing and sleep disruption may be more informative. Finally, we have not adjusted for the multiple phenotypes analyzed, because these OSA-associated quantitative phenotypes are significantly correlated.

The present study suggests the presence of multiple genetic determinants of the pathophysiological traits associated with OSA and suggests that two of the important phenotypes associated with OSA (AHI and BMI) are distinct traits with both shared and unshared genetic determinants. There are likely multiple genetic determinants of OSA, and these linkage analyses of quantitative phenotypes have identified several regions of interest on which to focus fine-mapping efforts. Evaluation of the regions of linkage within our study through use of additional STR markers will be required, and replication of our findings in other samples will be necessary. Investigation of candidate genes in the regions of linkage will also be necessary. If novel genetic determinants of OSA can be identified, important new insights into OSA pathophysiology—and, ultimately, treatment—could result.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ (for the NHLBI mammalian genotyping service)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for OSA [MIM 107650], obesity [MIM 601665], acid phosphatase 1 [MIM 171500], apolipoprotein B precursor [MIM 107730], proopiomelanocortin [MIM 176830], alpha-2B-adrenergic receptor [MIM 104260], neuropeptide Y [MIM 162640], vitamin D receptor [MIM 601769], insulin-like growth factor 1 [MIM 147440], and apolipoprotein E [MIM 107741])
UCSC Genome Bioinformatics, http://genome.cse.ucsc.edu/ (for Human Genome Working Draft "Golden Path")
University of Arizona/Channing Labs, Programs for Genomic Applications, http://pga.bwh.harvard.edu/ (for SNPper)

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