Brief Genetics Report

Quantitative Trait Loci on Chromosome 8q24 for Pancreatic β-Cell Function and 7q11 for Insulin Sensitivity in Obese Nondiabetic White and Black Families

Evidence From Genome-Wide Linkage Scans in the NHLBI Hypertension Genetic Epidemiology Network (HyperGEN) Study

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Genome-wide linkage scans were carried out using a multipoint variance components method in white and black families of the NHLBI Hypertension Genetic Epidemiology Network (HyperGEN) study to identify quantitative trait loci (QTLs) for pancreatic β-cell function and insulin sensitivity estimated through the newly released nonlinear computer version of homeostasis model assessment 2. Participants fasting <8 h, with diagnosed type 2 diabetes, or taking blood glucose or blood lipid–lowering medications were excluded. Both phenotypes were adjusted separately by race and sex for the effects of age, BMI, and field center before linkage scans using 370 microsatellite markers were performed. A total of 685 white families (1,180 sibpairs) and 773 black families (775 sibpairs) were evaluated as well as subsets including 267 obese white families (757 sibpairs) and 427 obese black families (599 sibpairs) identified through tree-linkage analyses using interacting covariates of age, sex, and BMI. For β-cell function in the obese white families, significant (logarithm of odds [LOD] score >3.6) evidence supporting linkages was detected on chromosome 8q24 at D8S1179 (135 cM, LOD score 4.2, empirical *P* = 0.002) and at D8S1128 (140 cM, LOD score 3.7, empirical *P* = 0.003). In addition, two regions supported linkage for insulin sensitivity index in the obese black families on chromosome 7q11 at D7S3046 (79 cM, LOD score 3.0, empirical *P* = 0.018) and on chromosome 6q26 at D6S1277 (173 cM, LOD score 3.0, empirical *P* = 0.018). Reducing clinical heterogeneity using obesity data and improved estimates of β-cell function and insulin sensitivity may have permitted identification of a QTL on chromosome 8q24 for β-cell function in the presence of estimated insulin resistance and a QTL on chromosome 7q11 for insulin sensitivity. These regions replicate previous reports for type 2 diabetes–associated traits. *Diabetes* 55:551–558, 2006

Defective pancreatic β-cell function (BCF) in the face of insulin resistance is a recognized hallmark of type 2 diabetes, where a β-cell defect prevents compensatory upregulation of insulin secretion (1). Quantitative estimates of BCF and insulin sensitivity may be obtained using well-established methods of stimulated euglycemic clamp and minimal model assessment. Less accurate methods, such as homeostasis model assessment (HOMA), also estimate BCF and insulin sensitivity. No single test is appropriate under all circumstances, but convenience and robustness make HOMA more appropriate for large population studies (2). The original model (HOMA1) was described in 1985 with a formula for approximate estimation (3). A more accurate nonlinear computer model (HOMA2) was described in 1998 (4), followed by the online release of a HOMA2 calculator in 2004 (www.OCDEM.ox.ac.uk). In view of its more precise physiological basis than that of HOMA1, HOMA2 is preferred (4) relative to the minimal model and other estimates (5).

Evidence of genetic components for BCF and insulin sensitivity has consistently been identified. Direct measures of glucose homeostasis using minimal model may

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BCF, β-cell function; FBPP, Family Blood Pressure Program; HOMA, homeostasis model assessment; HyperGEN, Hypertension Genetic Epidemiology Network; LOD, logarithm of odds; QTL, quantitative trait locus.

TABLE 1
Sample characteristics for overall and obese white and black families

	n	Age (years)	BMI (kg/m^2)	Glucose (mg/dl)	Insulin (μU/ml)	BCF (%)	Insulin sensitivity (%)
Whites							
Overall							
Men	451	54.2 ± 0.7	29.3 ± 0.2	97.9 ± 0.6	8.6 ± 0.3	84.9 ± 1.5	117.3 ± 2.7
Women	520	55.4 ± 0.6	30.1 ± 0.3	95.9 ± 0.6	8.3 ± 0.2	87.2 ± 1.5	122.7 ± 2.7
Obese							
Men	244	51.9 ± 0.9	30.9 ± 0.3	98.4 ± 0.8	9.7 ± 0.4	90.4 ± 2.3	108.2 ± 3.7
Women	308	53.3 ± 0.7	32.8 ± 0.5	96.0 ± 0.9	9.5 ± 0.4	93.0 ± 2.0	111.9 ± 3.4
Blacks							
Overall							
Men	375	46.2 ± 0.7	30.1 ± 0.3	97.8 ± 0.9	9.4 ± 0.3	91.0 ± 2.0	113.0 ± 3.1
Women	752	45.7 ± 0.5	33.5 ± 0.3	95.3 ± 0.7	10.9 ± 0.3	107.5 ± 1.5	95.9 ± 1.9
Obese							
Men	257	45.1 ± 0.8	31.5 ± 0.4	98.0 ± 1.1	10.1 ± 0.4	95.8 ± 2.5	105.5 ± 3.6
Women	551	44.8 ± 0.5	35.5 ± 0.3	96.2 ± 0.8	11.7 ± 0.3	111.4 ± 1.8	88.9 ± 2.1

Data are means ± SE.

have different genetic architecture versus the surrogate HOMA indexes (6). In recent years, a few genome-wide linkage scans of direct measures of glucose homeostasis became available in the FUSION (Finland-U.S. Investigation of Non–Insulin-Dependent Diabetes Mellitus Genetics) study (7), a hypertension and insulin resistance study in Hispanics (8), the HERITAGE (Health, Risk Factors, Exercise Training, and Genetics) Family Study (9–10), and the IRAS (Insulin Resistance Atherosclerosis Study) Family Study (11). In contrast, HOMA1 indexes derived from fasting glucose and insulin have been more widely used in genome-wide linkage scans. These studies include a genome-wide linkage scan in the Hypertension Genetic Epidemiology Network (HyperGEN) study (12) and a meta-analysis in the Family Blood Pressure Program (FBPP), which is comprised of the HyperGEN and three other component networks (13). In the current study, based on the white and black HyperGEN samples, both BCF and insulin sensitivity were estimated using HOMA2. To identify quantitative trait loci (QTLs) for these nonlinear measures of HOMA, we carried out genome-wide linkage scans in the entire sample as well as in obese subsets of families identified through tree-linkage analyses using interacting covariates of age, sex, and BMI.

RESEARCH DESIGN AND METHODS

The HyperGEN study population, design, and methods were described in detail elsewhere (14). In brief, HyperGEN sampled white and black sibships containing sibpairs with essential hypertension, and family members were recruited from five participating clinical centers located in Framingham, Massachusetts; Minneapolis, Minnesota; Salt Lake City, Utah; Forsyth County, North Carolina; and Birmingham, Alabama; respectively. A sibship was ascertained if it had at least two siblings whose blood pressure was ≥140/90 mmHg or who used antihypertension medications, with an age at diagnosis of <60 years. Family relationships were confirmed using the marker data, and pedigree errors were corrected with unrelated individuals excluded from the study. Also excluded were those with fasting times <8 h; those with fasting glucose and insulin concentrations out of the range of 1–25 mmol/l and 1–2,200 pmol/l, respectively (5); those with diagnosed type 2 diabetes; and those on lipid or blood glucose–lowering medications. Additionally, a large random sample of unrelated individuals was recruited, regardless of hypertension status, from the same source population as the hypertensive sibling population. Allele frequencies were estimated separately in whites and blacks using marker allele frequencies derived from the randomly selected subgroup of whites and blacks. After covariate effect correction using the random sample as well as integration of marker information, a total of 971 subjects comprising 1,180 sibpairs from 685 white families and a total of 1,127 subjects comprising 775 sibpairs from 773 black families were available for the current analysis (Table 1). The study protocol was approved by the institutional review boards of each participating site, and written informed consent was obtained from each participant.

Pancreatic BCF and insulin sensitivity estimates were made available using the newly released HOMA2 calculator (3–5). Raw phenotypes were skewed and approximately normalized using a squared-root transformation for BCF and a log transformation for insulin resistance. The random setting of HyperGEN data was used for data adjustment procedures. Before genetic analysis, each phenotype was corrected for the effects of age, age^2, age^3, BMI, and field-center separately by sex within race in both the mean and the variance using a stepwise multiple regression procedure. For each of the regressions, only terms significant at the 5% level were retained. Outliers were defined as departures at least 4 SDs from the mean and at least 1 SD from the adjacent observation and set to "missing" in the current study. Finally, each of the adjusted variables was standardized (mean ± SD: 0 ± 1) separately by sex within race.

Morning fasting serum samples from all study subjects were collected in a resting state. Briefly, these samples were measured in duplicate for fasting serum glucose concentrations using Elan Glucose reagent and for fasting serum insulin concentrations on an automated immunoassay instrument with ultra-sensitive insulin kit from Beckman Coulter (Fullerton, CA) (15). Detailed measurement methods have been described elsewhere (12). For the glucose assay, the sensitive range is 2–450 mg/dl with the observed detection limit of 1.02 mg/dl. The sensitivity and dynamic range of the insulin assay are 0.03 and 0.03–300 mU/l, respectively, with 0 cross-reactivity with proinsulin and C-peptide, 30% with bovine insulin, and 97% with porcine insulin (12).

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, Wisconsin (http://research.marshfieldclinic.org/genetics), for genotyping. In this study, a total of 370 highly polymorphic microsatellite markers were used, which has an average heterozygosity of 80%, an average intermarker spacing of 10 cM, and a 95% coverage of the entire human genome.

Multipoint linkage analyses were performed using the variance components model as implemented in the computer program SEGPATH (16,17). Under the variance components model, a phenotype is under the influence of the additive effects of a trait locus (g), a residual familial background modeled as a pseudo-polygenic component (G_R), and a residual nonfamilial component (r). The effects of the trait locus and the pseudo-polygenic component on the phenotype are quantified by the heritabilities h^2_g and h^2_r, respectively. Allele-sharing probabilities at each marker location for each sibpair were estimated using the multipoint approach in the computer program MAPMAKER/SIBS (18) and were input to the SEGPATH model. Other parameters in the model include spouse resemblance (u), additional sibling resemblance (b), and the phenotype means and variances. The linkage hypothesis is tested by restricting $h^2_g = 0$. A likelihood ratio test contrasting the null versus the alternative hypotheses is asymptotically distributed as a 50:50 mixture of a χ^2 with 1 d.f. (degree of freedom) and a point mass at 0 (19), and the LOD score is computed as $\chi^2/(2 \times \log_e 10)$.

Aggregate samples may be subdivided into homogeneous subgroups, within some of which linkage signals may be enriched and power potentially enhanced in QTL mapping. Rapid assessment of the evidence can be accomplished by using Haseman-Elston regression model (20,21). A tree-based

TABLE 2
Promising (LOD scores >1.75) multipoint genome-wide linkage scan results for BCF

	Chromosome	Location (cM)	Marker	LOD score	*P*	Empirical *P*
Whites	2q22	86.82	D2S1799	2.10	0.001	0.188
	8q24	135.08	D8S1179	1.90	0.002	0.188
Blacks	7q11	90.95	D7S2204	1.77	0.002	0.127
	8p22	26.43	D8S1106	2.29	0.001	0.106
	12q24	125.31	D12S2070	2.00	0.001	0.106
	13q12	8.87	D13S787	1.93	0.001	0.106
Obese whites	8q24	135.08	D8S1179	4.20	0.000	0.002

recursive partitioning method, based on interacting covariates, was used to identify such subgroups. It is implemented in SPLUS computer routine RPART (22), which has been modified for sibpair linkage analysis (23,24). At each step, sibpair data are partitioned into two mutually exclusive subgroups, which are determined by splitting the pool at the optimal cut point of the covariate observed in the sibpair that best partitions the linkage evidence. Also at each step, the Haseman-Elston regression is evaluated in each possible partition of the data for each eligible covariate. The split producing the greatest contrast in the Haseman-Elston regression slope is chosen. Further splits at each child node are evaluated recursively until a prespecified complexity is reached. The tree is then pruned back to minimize noise, eliminating splits where the residual sum of squares of the Haseman-Elston regression model is within 1 SE of that in the final step. Since genotype-covariate interactions provide the basis for the tree-linkage analysis to identify a linked subset of families, the identification of such a group can be considered as evidence for presence of such interactions.

Let $Y_i = (y_{i1} - Y)(y_{i2} - Y)$ be the "response" variable, where Y is the grand mean of the phenotype for the ith sibpair ($i = 1,\ldots, n$) (21). In this model, $y_i = \beta_0 + \beta_1 M_i + \epsilon_1$, where M_i denotes the proportion of alleles at the marker shared identical-by-descent by the ith sibpair using MAPMAKER/SIBS (18) and the coefficient β_1 indicates the strength of the linkage of the marker to the locus. The test of $\beta_1 = 0$ is a test for absence of linkage. Covariate information on sibs must be interpreted at the level of sibpairs. Several different functions of the covariate values for the sibpair can be considered. They include, for example, the mean, minimum, and maximum for quantitative covariates and concordance and/or discordance for each outcome for qualitative covariates. To split a node, the model sum of squares is calculated for $Y = \beta_0 + \beta_1 M_i + \beta_2 M_i\, I(X_j > c_j) + \beta_3 M_i\, I(X_j \le c_j)$ over all covariates X_j and all cut points c_j, where $I(.)$ is the logical indicator function (1 if the expression is true and 0 if false). The covariate and its respective cut point leading to the smallest mean square error of the model are selected for splitting the sample. The algorithm is recursively applied to each child node obtained from the split. In the end, all terminal nodes of the tree are classified into the "linkage" group (the Haseman-Elston regression slopes $\beta > 0$, $P < 0.05$) and the remaining group. The identified sibpairs and the associated families from the linkage group can be analyzed further.

A false discovery rate (25) was used as a measure of global error for multiple testing simulations, i.e., the expected proportion of false rejections of the null hypothesis among the total number of rejections. False discovery rate for each trait was estimated separately by white and black data using the SAS package, and the associated empirical *P* values were reported in Table 2 for BCF and in Table 3 for insulin sensitivity.

RESULTS

Sample sizes and data characteristics for whites and blacks are given separately by sex in Table 1. In general, ~70% of family members were diagnosed with essential hypertension, and ~30% of subjects had pre-diabetes (fasting glucose 100–125 mg/dl) or suspected diabetes (fasting glucose ≥126 mg/dl). The percentage of clinically defined obese subjects (BMI ≥30 kg/m^2) was 42% in whites versus 57% in blacks. Overall, heritability estimates ranged from 30 to 60%, with higher estimates for BCF in whites and for insulin sensitivity in blacks. Promising regions supporting linkage (LOD scores >1.75) (26) from the multipoint genome-wide linkage scans include chromosomes 2q22 and 8q24 in whites and 7q11, 8p22, 12q24, and 13q12 in blacks for BCF. Details are given in Table 2 and also depicted in Fig. 1. Promising linkages for insulin sensitivity include chromosome 17q25 in whites and 6q26, 7q11, and 12q12 in blacks, and their complete results are given in Table 3 and Fig. 2. Tree-linkage analysis resulted in selected promising regions on chromosomes 2, 6, 7, 8, 12, and 13 that were similar to those from the obese families (but not exactly identical), leading to the identification of the obese subsample with BMI >33 kg/m^2 (e.g., D8S1179, mean BMI >33.41, Haseman-Elston regression slope $\beta = 1.15$, $P < 0.05$; D13S787, mean BMI >34.73, $\beta = 1.05$, $P < 0.05$). Data characteristics of the tree-identified obese subgroup of families are given in Table 1, and promising linkages arising from the tree-identified obese subgroup of families are also given in Tables 2 and 3 with complete linkage scan results depicted in Figs. 1 and 2. In general, striking linkages with LOD scores ≥3.0 on chromosome 8q24 (79–91 cM) for BCF in obese whites and on chromosomes 7q11 (79–98 cM) and 6q26 (166–173 cM) for insulin sensitivity in blacks were observed.

DISCUSSION

Genome-wide linkage scans aimed at identifying QTLs for type 2 diabetes and its associated traits are accumulating. However, findings seldom replicate across studies. Because type 2 diabetes represents a complex disorder with substantial clinical and genetic heterogeneity, efforts to define and identify genetically homogeneous subsamples

TABLE 3
Promising (LOD scores >1.75) multipoint genome-wide linkage scan results for insulin sensitivity

	Chromosome	Location (cM)	Marker	LOD score	*P*	Empirical *P*
Whites	17q25	116.86	D7S784	1.74	0.002	0.576
Blacks	6q26	173.31	D6S1277	2.81	0.000	0.059
	7q11	78.65	D7S3046	2.11	0.001	0.165
	12q12	56.25	D12S1301	1.76	0.002	0.165
Obese blacks	2q35	215.78	D2S434	2.03	0.001	0.058
	6q26	173.31	D6S1277	2.99	0.000	0.018
	7q11	78.65	D7S3046	3.01	0.000	0.018
	9q32	120.04	D9S930	2.32	0.001	0.040
	12q12	56.25	D12S1301	2.86	0.000	0.018

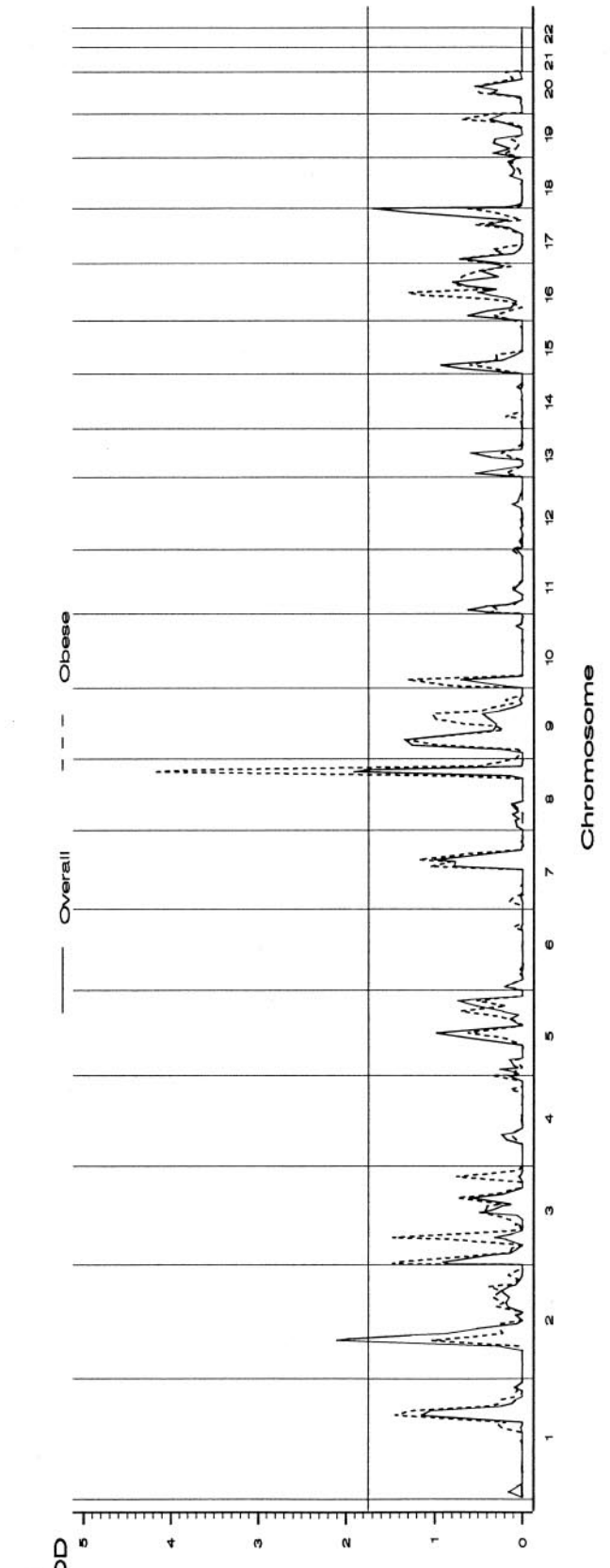

FIG. 1. Genome-wide linkage scan results for BCF in overall (solid line) and obese (dashed line) whites (*A*) and blacks (*B*). LOD score of the reference line is 1.75 for promising linkages.

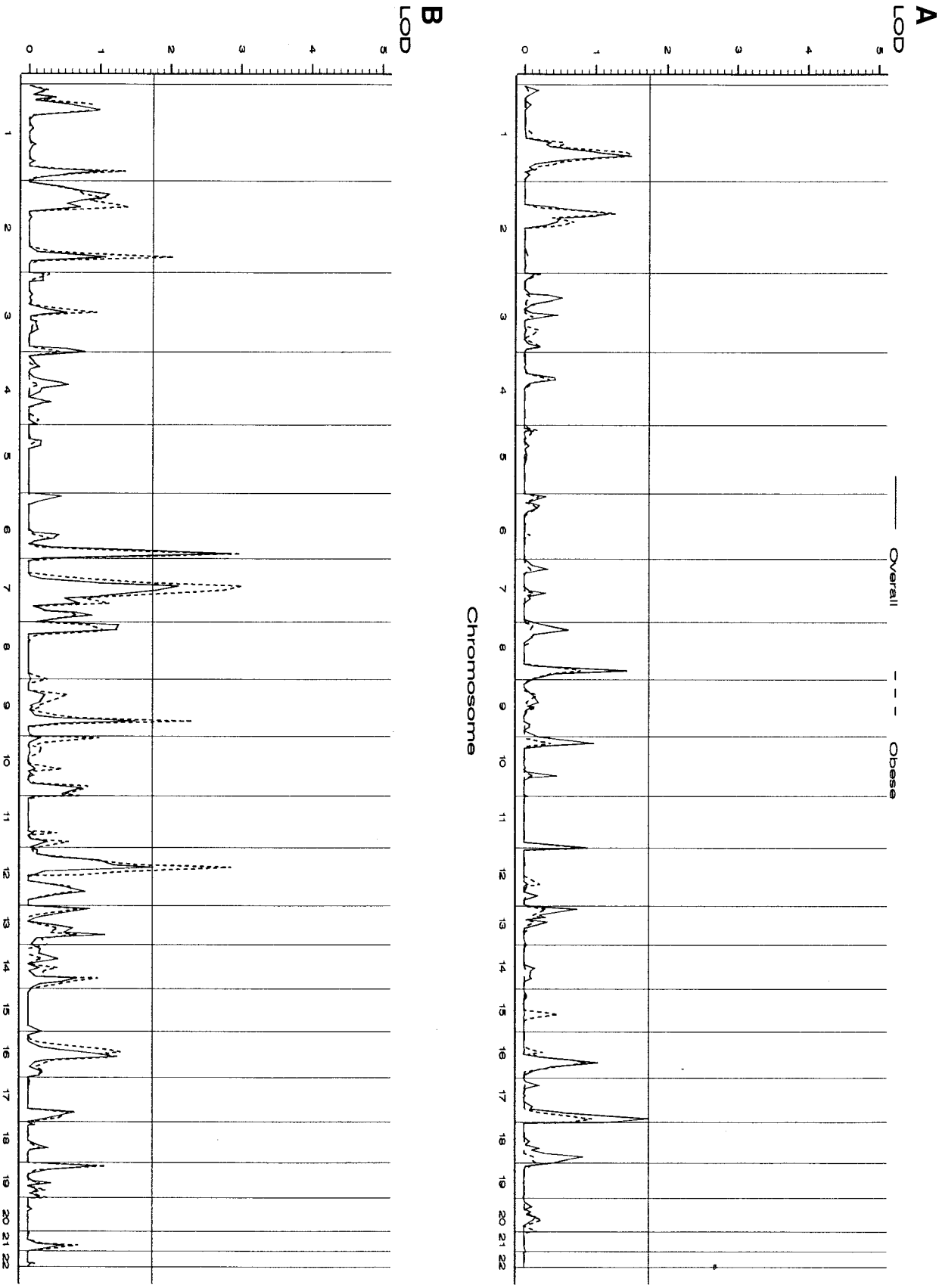

FIG. 2. Genome-wide linkage scan results for insulin sensitivity in overall (solid line) and obese (dashed line) whites (*A*) and blacks (*B*). LOD score of the reference line is 1.75 for promising linkages.

using clinical characteristics and pre-diabetes phenotypes, before the frank development of overt type 2 diabetes, may enhance gene discovery. These analyses in a biracial cohort of HyperGEN families are unique. First, this study is highly enriched with hypertensive subjects, offering a potentially more homogeneous sample (subphenotype) (13). Second, more precise estimates of BCF and insulin sensitivity from HOMA2 were used. Because concentration ranges have been set for fasting insulin (1–2,200 pmol/l) and glucose (1–25 mmol/l) (5), resulting in slightly reduced sample sizes by excluding some extreme values whose underlying genetic basis might be different, both BCF and insulin sensitivity appear to have been actually estimated in more homogeneous data settings. Finally, genome-wide linkage scans in obese families might further gene discovery because insulin resistance and type 2 diabetes frequently coexist with obesity, with or without hypertension (largely through underlying obesity) (27). The obese families in this study, including those with at least one family member whose BMI was >33 kg/m^2, were identified through tree-linkage analysis using selected interacting covariates of age, sex, and BMI.

The linkage on chromosome 8q24 (135.1–139.5 cM) for BCF in whites was significant (LOD score >3.6) (18), with support of linkage evidence increased from LOD scores = 1.9 in the entire set of families to LOD score = 4.2 in the set of obese families. Though the linkage scan results for insulin sensitivity in the complete data were not impressive, modest support for linkage (LOD score = 1.4) at the same location was observed (Fig. 2*A*). Having such an observation is not surprising, since BCF and insulin sensitivity are recognized as associated traits. Clinically, β-cell dysfunction is an early feature of type 2 diabetes, while insulin resistance is a fundamental trigger for the development of type 2 diabetes (28). Compensatory BCF increase is often the initial β-cell response in the presence of insulin resistance, and according to the type 2 diabetes "2-hit" phenomenon proposed by Bergman et al. (1), insulin resistance is accompanied by a β-cell defect preventing compensatory upregulation of insulin secretion. In this study, the region supporting linkage for BCF in whites was not replicated in blacks. It should be noted that participants in the black families had more women and younger age, greater BMI, higher fasting insulin concentration (with compatible fasting glucose concentration), lower insulin sensitivity, and higher BCF than participants in the white families. In addition, genetic heterogeneity and clinical heterogeneity for this "pre-diabetes phenotype" could in part account for the difficulty in replicating linkage evidence across races. Thus, this identified region on chromosome 8q24 for BCF observed in the obese hypertensive families may be population-specific to whites, with a different (or overlapping) set of genes contributing to variation in BCF in blacks.

Genome-wide linkage scans of stimulated insulin sensitivity and BCF parameters using euglycemic clamp (8) or minimal model assessment (7,9–11) have been recently reported. However, the genetic basis of these parameters is likely to be different from the basal surrogate estimates through HOMA (6). Linkage scans using the HOMA2 (4–5) estimates have not been reported. Most recently, a genome-wide linkage scan of HOMA1 insulin resistance index in the HyperGEN (12) and a meta-analysis in the FBPP (13) did not reveal promising linkage evidence at this location. The region of interest in our study (chromosome 8q24) has been shown to be linked to type 2 diabetes in a U.K. population (D8S284, 143.82 cM) (29), to type 1 diabetes in a Dutch population (D8S1128, 139.53 cM) (30), to unesterified HDL cholesterol in Mexican Americans (D8S1128) (31), to LDL cholesterol and apolipoprotein B-100 in sedentary whites (D8S1774, 137.9 cM) (32), and to systolic blood pressure in sedentary blacks (D8S1179, 135.1 cM) (33). No prominent candidate genes at this locus have been proposed, but these clusters of associated linkage results across studies and populations suggest the presence of at least one QTL of relevance to phenotypes of the metabolic syndrome.

Chromosomes 7q11 (78–91 cM) and 6q26 (166–173 cM) are two regions supporting linkage for insulin sensitivity in both the entire family collection and in the obese black families. Promising linkages for BCF was also observed at the same region in the obese white families (Fig. 1*B*). In addition, we observed that mean insulin sensitivity in the black families was noticeably lower than that in the white families (Table 1), implying that blacks are more insulin resistant. The linkage on chromosome 7q11 has been replicated in several other studies (13). The glycogen-associated regulatory subunit of type 1 protein phosphatase (*PPP1R3*) gene resides in this region. This gene product binds to muscle glycogen with high affinity, thereby enhancing dephosphorylation of glycogen-bound substrates for protein phosphatase-1 such as glycogen synthase and glycogen phosphorylase kinase (OMIM 600917). Consistently, this gene product has been associated with insulin sensitivity (34) and severe insulin resistance, glycemia variation, and type 2 diabetes (35–37). Two previous studies have also observed linkage near the paraoxonase (*PON*) gene locus for disposition index exercise training response (109 cM, LOD score = 1.4) in the HERITAGE Family Study (10) and for type 2 diabetes (114 cM, *Z* score = 1.9) in Pima Indians (38). The region of linkage insulin sensitivity as estimated by HOMA2 appears to be different from those for the insulin resistance index estimated by HOMA1 (found at more distal locations of the long arm, 182 cM, LOD score = 1.7) in a recent scan in the HyperGEN (12) and in a meta-analysis (163–174 cM, LOD scores = 2.6–3.2) in the FBPP (13). HOMA2 requires fasting insulin and glucose concentrations within ranges of 1–2,200 pmol/l and 1–25 mmol/l, respectively. The HOMA2 results in slightly reduced sample sizes but likely better estimates from a more homogeneous sample. As for the locus on chromosome 6q26, a recent linkage scan in a Hong Kong Chinese population mapped HOMA1 insulin resistance index at 119 cM (LOD score = 3.0) (39), seemingly a different locus from our current insulin sensitivity linkage peak in obese blacks. For type 2 diabetes and associated trait loci around this region (166–173 cM), linkage evidence has been obtained in African Americans for type 2 diabetes (40), in African Americans in the FBPP (41), and in a meta-analysis of six linkage scans (42) for hypertension. There is growing interest in assessing whether this locus harbors pleiotropic genes for type 2 diabetes, obesity, and hypertension with interactions among these genes and complex diseases appropriately measured in future genetic studies. Replications by other independent studies and fine-scale mappings on both chromosomes 7q11 and 6q26 for insulin sensitivity or insulin resistance are clearly warranted.

In conclusion, pancreatic BCF and insulin sensitivity estimated through HOMA2 were explored for the first time in multipoint variance component genome-wide linkage scans. Based on the linkage evidence in obese and hyper-

tensive nondiabetic families, the QTLs on chromosomes 8q24 and 7q11, which are located in regions previously identified as harboring type 2 diabetes–associated genes, may govern insulin sensitivity and insulin secretion in the presence of insulin resistance before development of overt type 2 diabetes. Follow-up fine-scale mapping around these loci and well-designed candidate gene studies, in particular, are strongly encouraged.

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