Brief Genetics Report

A Genome Scan for Fasting Insulin and Fasting Glucose Identifies a Quantitative Trait Locus on Chromosome 17p

The Insulin Resistance Atherosclerosis Study (IRAS) Family Study

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Plasma insulin and glucose concentrations are important quantitative phenotypes related to diabetes and the metabolic syndrome. Reports purporting to identify quantitative trait loci (QTLs) that contribute to the variation in fasting insulin and glucose concentrations are discrepant. As part of the Insulin Resistance Atherosclerosis Study (IRAS) Family Study, a genome scan was performed in African-American ($n = 42$) and Hispanic ($n = 90$) extended families to identify regions that may contain positional candidate genes for fasting insulin and fasting glucose ($n = 1{,}604$ subjects). There was significant evidence for linkage of fasting insulin to the short arm of chromosome 17 (logarithm of odds [LOD] = 3.30; 54 cM between D17S1294 and D17S1299, $P = 1.0 \times 10^{-4}$). The strongest evidence for linkage over all pedigrees for fasting glucose was also observed in this region (LOD = 1.44; 58 cM, $P = 9.9 \times 10^{-3}$). The results of this study provide impetus for future positional cloning of QTLs regulating insulin and glucose levels. Identifying genes in these regions should provide insight into the nature of genetic factors regulating plasma glucose and insulin concentrations. *Diabetes* 54: 290–295, 2005

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Received for publication 6 July 2004 and accepted in revised form 8 October 2004.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

HETE, hydroxyeicosatetraenoic; HODE, hydroxyoctadecadienoic; IBD, identity by descent; IRAS, Insulin Resistance Atherosclerosis Study; LOD, logarithm of odds; QTL, quantitative trait locus.

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Both insulin resistance and β-cell dysfunction are predictors of type 2 diabetes (1–5). The discovery of genes that modify the risk of type 2 diabetes, as mediated through glucose homeostatic pathways, has been challenged by limited statistical power. These problems may be due to the underlying heterogeneity of the phenotype (type 2 diabetes), the complex interaction between genetic and environmental determinants for each trait, and the relatively low signal-to-noise ratio of the genetic component, as indicated by a genetic risk ratio (λ_S) for type 2 diabetes of <2 (6). One approach to gaining a better understanding of the genetics of type 2 diabetes is to study the genetic basis of intermediate quantitative traits that establish an individual's genetic susceptibility. The most common phenotypes used for this purpose have focused on fasting insulin and fasting glucose. In this study, a genome scan was conducted for fasting insulin and fasting glucose, two phenotypes that are related to insulin resistance and type 2 diabetes. The results of this study have identified regions of the genome that contain potential quantitative trait loci (QTLs) that contribute to the variation in fasting insulin and fasting glucose in extended pedigrees of Hispanic and African-American descent.

A total of 132 extended families (42 were African American from Los Angeles, 60 were Hispanic from San Antonio, and 30 were Hispanic from San Luis Valley), representing 2,019 individuals with phenotypic and genetic data, were included in these analyses. Fasting glucose and fasting insulin were coded as "unknown" for subjects with diabetes, although genotypic data were used. Given the

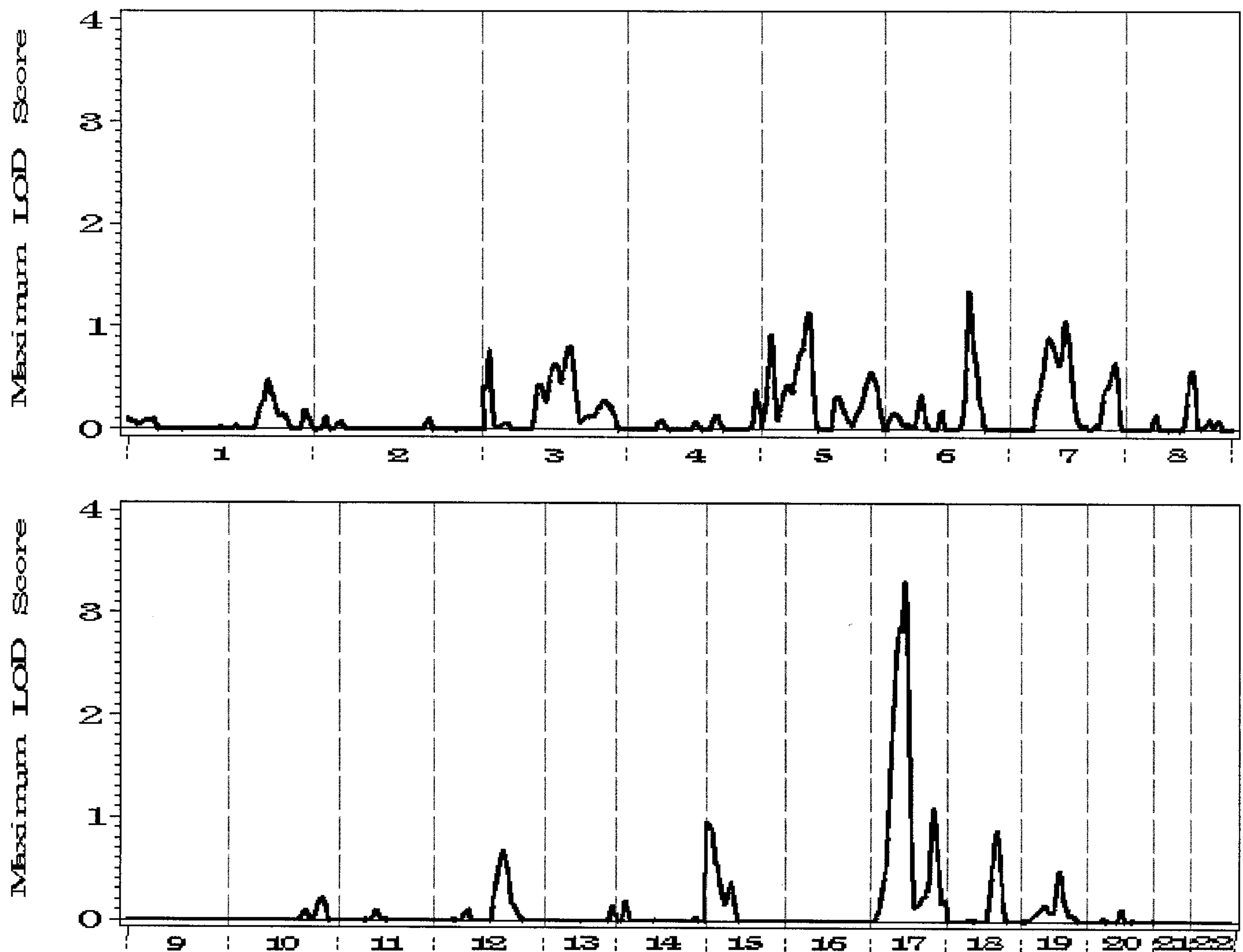

FIG. 1. Results of the genome-wide scan of fasting insulin levels in the IRAS Family Study.

large family sizes, there were 2,675 sibling pairs and 5,176 avuncular pairs of relatives contributing to linkage. Participating family members were on average 43 years of age (range 18–81) and 57% female. Mean fasting insulin ±SD was 15.1 ± 11.9 μU/ml, and mean fasting glucose was 93.9 ± 9.8 mg/dl. Mean BMI was 29.1 ± 6.3 kg/m^2, and 12.7% of the participants had type 2 diabetes. There were no significant differences in these characteristics across the three centers.

The heritability (h^2) of fasting insulin in the entire sample was 0.17 ± 0.05 ($P < 0.001$). The covariates (age, sex, BMI, and center/ethnicity) accounted for 23% of the variation in fasting insulin. The heritability (h^2) of fasting glucose in the entire sample was 0.27 ± 0.06 ($P < 0.001$). Covariates (age, sex, BMI, and center/ethnicity) accounted for 22% of the variation in fasting glucose.

Results of multipoint nonparametric linkage analysis for fasting insulin are shown in Fig. 1. Over all centers, the strongest evidence for linkage to fasting insulin was found on chromosome 17 (Fig. 2), with logarithm of odds (LOD) = 3.30 at 54 cM, between flanking markers D17S1294 and D17S1299, and support interval between positions 38 cM and 59 cM ($P = 1.0 \times 10^{-4}$). The empirical LOD = 3.16 (empirical $P = 1.0 \times 10^{-4}$) and occurred at the same location. Both Hispanic (LOD = 2.20, position 55 cM) and African-American (LOD = 1.16, position 50 cM) families contributed to the evidence for linkage at this location. The only other location in the genome with LOD >1.58 (approximately nominal genome-wide 1% significance) occurred on chromosome 6p in the San Luis Valley sample (LOD = 1.87 at 20 cM, support interval 6 cM–59 cM, $P = 0.003$, between markers ATTT030 and D6S2434). Detailed information for all chromosomes can be found in online appendix Table 1 (available from http//diabetes.diabetesjournals.org).

Over all centers, the strongest evidence for linkage to fasting glucose (Fig. 3) was also found on chromosome 17 (LOD = 1.44 at position 58 cM, support interval 36 cM–68 cM, $P = 9.9 \times 10^{-3}$). The empirical LOD = 1.44 (empirical $P = 5.3 \times 10^{-3}$) and supports evidence for linkage in the same location as fasting insulin. No other location in either the entire family set or in ethnic/center-specific subsets had LOD >1.58 (online appendix Table 2). In the Hispanic set of families, there was evidence of linkage to fasting glucose on chromosome 1q (LOD = 1.44, $P = 0.010$) in a region previously reported to support linkage for metabolic syndrome in the Insulin Resistance Atherosclerosis Study (IRAS) Family Study (7).

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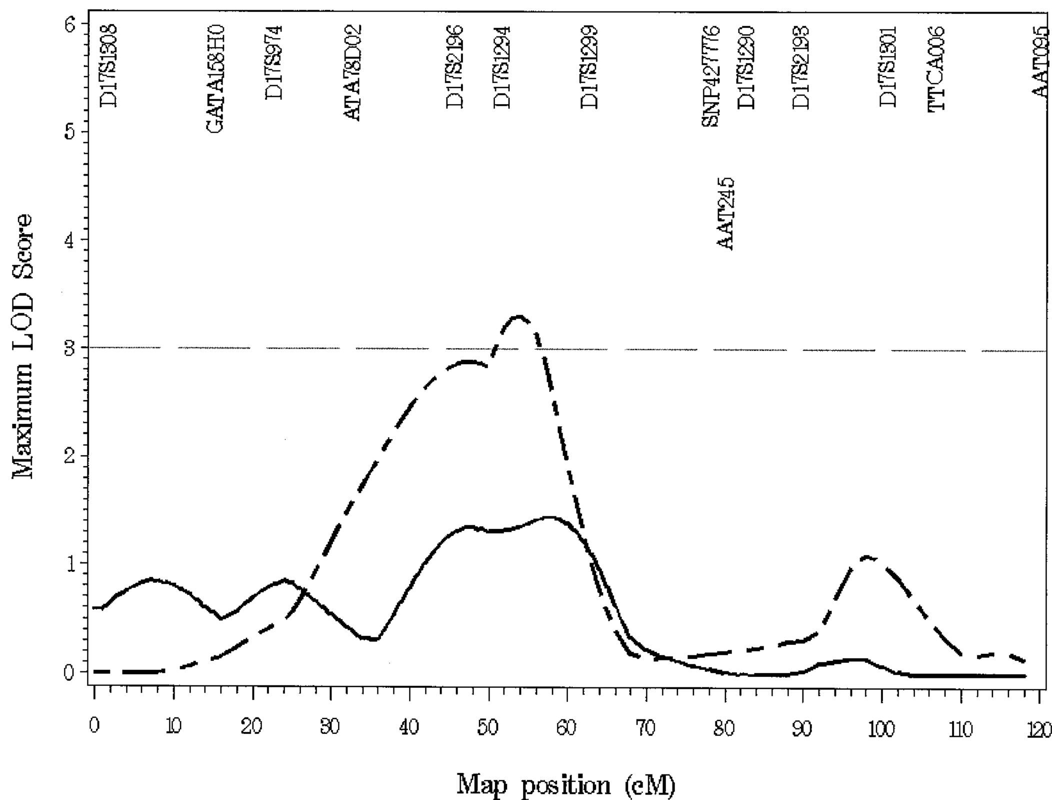

FIG. 2. Linkage of fasting insulin (– — –) and fasting glucose (——) levels to chromosome 17.

Genome scan microsatellite markers on chromosome 17 were examined for association with fasting insulin and fasting glucose using a generalized estimating equation (GEE) approach. Both allelic and genotypic associations were tested, with each phenotype adjusted for age, sex, clinic, and BMI. No microsatellite marker on chromosome 17 had a $P < 0.01$ for global allelic or genotypic association.

In this study, the primary location for the existence of a putative QTL influencing variation in fasting insulin and fasting glucose occurs on the short arm of human chromosome 17. This region of the genome has not been a focus of evaluation for these quantitative traits, although several previous studies have noted modest evidence for linkage to diabetes-associated phenotypes in this region.

Several reports of linkage to fasting insulin have been reported. In the Framingham Offspring Study (8), the strongest linkage (not BMI adjusted) for fasting insulin occurred on chromosome 11p (LOD = 2.43 at D11S2002); however, there was evidence of linkage on chromosome 17p (LOD = 1.80 at D17S1299 at 60 cM). Moreover, when fasting insulin was adjusted for BMI, the evidence on 17p increased to LOD = 2.2 at a similar location as that observed in the IRAS Family Study. The Finland-United States Investigation of Non-insulin-dependent Diabetes Mellitus Genetics (FUSION) (9) investigators reported linkage with fasting insulin (age and sex but not BMI adjusted) on chromosome 17p (LOD = 2.97, position 9 cM), although to a region more telomeric than we report. A cluster of glucose homeostasis phenotypes map to this same general location in FUSION, including 2-h glucose, 2-h insulin, $S_{I(EST)}$ (the index for insulin resistance), and IR_I (the empirical index of insulin secretion). In a study of hypertensive Hispanic families (10), evidence of linkage for fasting insulin was observed on chromosome 7q (LOD = 3.36 at D7S3061) and 16p (LOD = 1.77 at D16S3396). In the same population, homeostasis model assessment, which is a function of fasting insulin and fasting glucose, provided evidence for linkage to chromosome 17p (LOD = 1.66 near D17S2193 at 80 cM). However, little evidence was found for linkage of fasting insulin or fasting glucose to 17p in several other genome scans, including the Pima Indians (11), the San Antonio Heart Study (12), and the HERITAGE Family Study (13–14).

This region (support interval) on human chromosome 17p that has been observed to contain a possible QTL for fasting insulin and other glucose homeostasis phenotypes contains several candidate genes (Fig. 4). These candidates involve signal transduction (glucagon-like peptide 2 receptor [GLP2R]), signal transduction (mitogen-activated protein kinases [MAPK] MAP2K4 and MAP2K3; and signal transduction and activation of transcription [STAT]5A and STAT5B), and the neuropeptide pancreatic polypeptide 2 (PPY2). Two strong positional candidate genes include GLUT4 (SLC2A4), the primary insulin-responsive glucose transporter in skeletal muscle, and complement C1q receptor protein (C1QBP), which is expressed in the endothelium, smooth muscle, and hepatocytes. Previously, evidence of epistatic interaction of a region on chromosome 3q (containing components of the metabolic syndrome) with plasma leptin on chromosome 17p was shown (15).

Another strong candidate pathway involves two members of the lipoxygenase (LO) gene family, ALOX12 and

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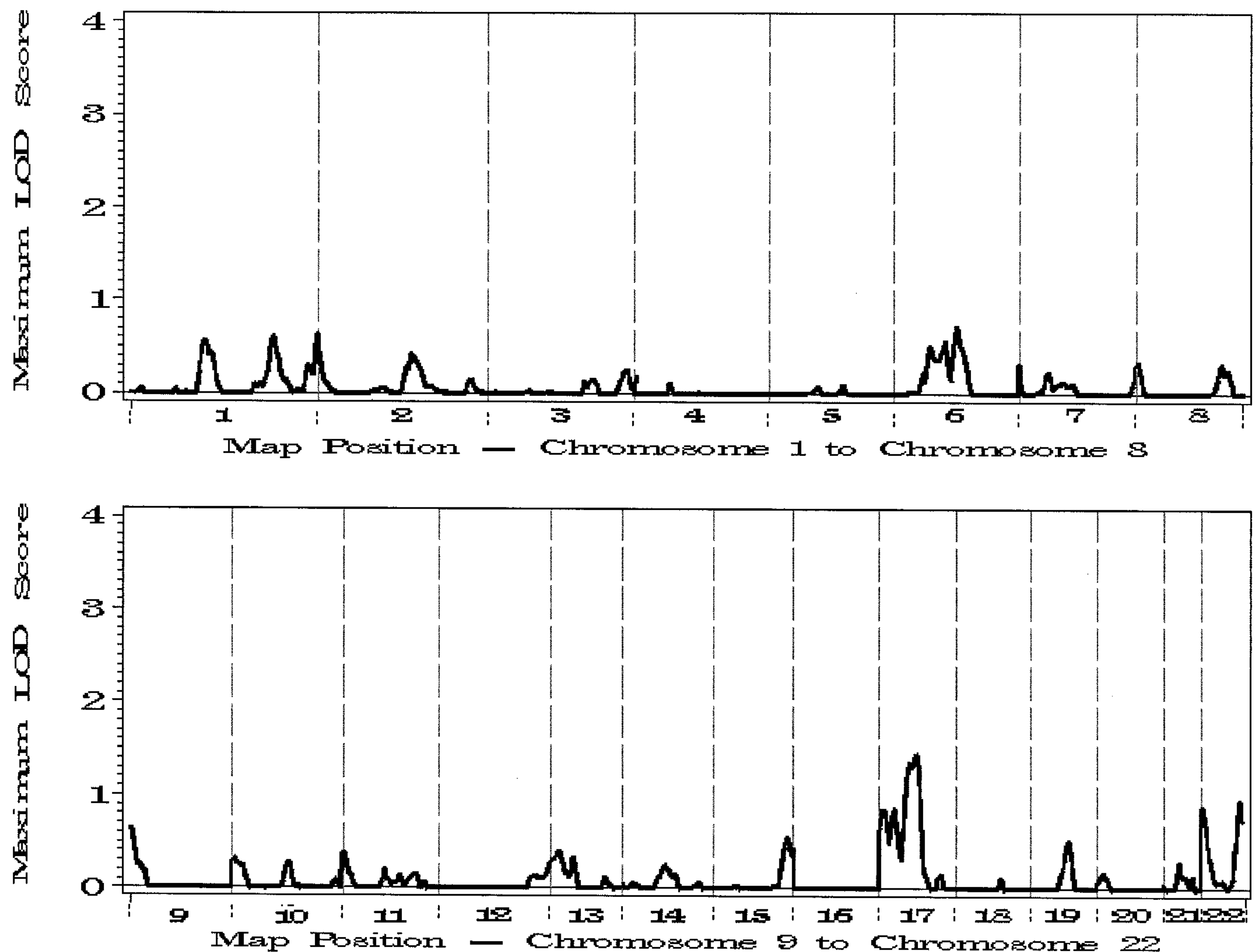

FIG. 3. Results of the genome-wide scan of fasting glucose levels in the IRAS Family Study.

ALOX15, whose products are localized in cells of the vessel wall, and generate eicosanoids, including 12-S- and 15-S-hydroxyeicosatetraenoic (HETE) acid and 13-S-hydroxyoctadecadienoic (HODE) acid. HETEs and HODEs act as second messengers in the vasculature to modulate inflammation (16). 12/15LO-deficient mice are resistant to the development of streptozotocin-induced diabetes (17), and increased 12/15LO activity occurs in diabetic *db/db* mice (18). Human aortic endothelial cells cultured chronically in elevated glucose produce significant elevations in 12SHETE and 13SHODE due to upregulation of the 12/15LO enzyme (18). In human diabetes, there is increased urinary excretion of 12SHETE together with possible shunting of arachidonic acid to the 12/15LO pathway (19). There is additional evidence to support a role for 12/15LO in the development of diabetes, as increased eicosanoid production has been reported in platelets of type 2 diabetic patients, leading to impaired antioxidant defense (20). The 12/15LO enzymes and components of the 12/15 LO pathway may contribute to the vascular complications of diabetes. In mouse models, deletion of the murine ALOX12 gene delays onset of atherosclerosis, and overexpression of the ALOX12 gene results in enhanced fatty streak formation in aorta (16,21). Thus, the 12/15LO pathway may also be a major contributor to the vascular complications observed in diabetes.

In summary, the results of our genome scan of fasting insulin and fasting glucose suggest that there may be common and distinct QTLs that influence parts of the biological pathway leading to insulin resistance and consequences of aberrant metabolism (including type 2 diabetes). A major unresolved question is the relationship between these factors. Genes that contribute to a strong candidate pathway (lipoxygenase) for type 2 diabetes and vascular complications of diabetes (12/15LO) may express effects through their control of variation in insulin and glucose levels. Relatively little is known about the role of these genetic factors and the role of these factors on inflammation, glucose metabolism, and the development of clinical disease.

RESEARCH DESIGN AND METHODS

The IRAS Family Study was designed to study the genetic epidemiology of insulin resistance and abdominal adiposity (22). Three clinical sites recruited and examined members of large families of Hispanic (San Antonio, TX, and San Luis Valley, CO) or African-American ethnicity (Los Angeles, CA) over a 2.5-year period (2000–2002). Potential probands were identified from the parent study, IRAS (23), a community-based study composed of approximately equal frequency of diabetic, impaired glucose tolerant, and normal

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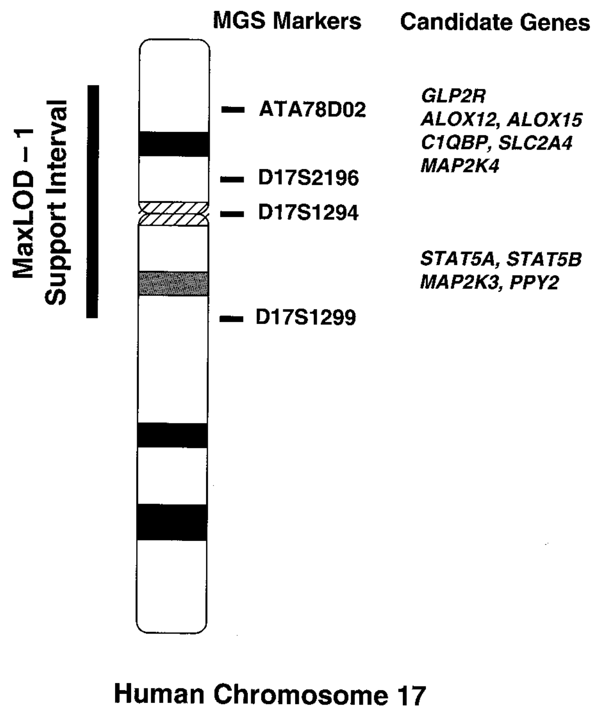

FIG. 4. Human chromosome 17, including the support interval for fasting insulin and fasting glucose and glucose homeostasis candidate genes.

glucose tolerant individuals. Probands in the IRAS reporting large family sizes were invited to participate with their relatives in the family study. Ascertainment was also supplemented with non-IRAS families, recruited from the general population. Ascertainment of families was not based on BMI or glucose tolerance status. The 132 pedigrees represent 66 families from a previous report on glucose homeostasis phenotypes (24), and 66 additional families were ascertained using the same criteria from the same centers.

Genotyping. Whole blood obtained from each IRAS Family Study participant was frozen and stored at −70°C and then shipped in batches to the Molecular Genetics Laboratory at Wake Forest University School of Medicine. Genomic DNA was extracted from the blood samples and stored. A genome scan consisting of markers at ~9 cM intervals was performed in the 132 families (in two batches of 66 families) by the NHLBI Mammalian Genotyping Service (Marshfield, WI).

QTL linkage analysis. Before performance of linkage analysis, all genetic data and family structure information were subjected to analyses to identify potential genetic marker problems and incorrect relationship assignment using PedCheck (25) and PREST software (26). Problems related to single-marker genotypes were resolved by recoding the genotype to unknown status. Nonpaternity was resolved by changing the pedigree structure to that most likely, then repeating the analysis. All analyses are based on the natural log transformation of fasting insulin and fasting glucose to better approximate conditional normality and to minimize heterogeneity of variance. For individuals with diabetes (by self-report or by fasting glucose >126 mg/dl), phenotypic values for fasting glucose and fasting insulin were coded as "unknown," although genotypic data were used for purposes of identity-by-descent (IBD) estimation. There were no outliers detected for either fasting glucose or fasting insulin with respect to BMI (after elimination of diabetic subjects).

Heritability (h^2) was estimated for the entire sample using age, sex, BMI, and center (San Antonio, San Luis Valley, and Los Angeles) as covariates. Within the Hispanic ethnic group, the models included age, sex, BMI, and center (San Antonio and San Luis Valley). For African Americans (ascertained only at the Los Angeles center), models included age, sex, and BMI as covariates. Estimation of residual heritability (that after the covariate effects were removed) was performed using the SOLAR software package (27). The heritability of fasting insulin and fasting glucose was determined as the ratio of polygenic to total phenotypic variance. Tests of significance between heritability estimates were conducted using likelihood ratio tests.

Determination of the support for linkage for fasting insulin and fasting glucose uses information from all possible pedigree relationships simultaneously based on the expected covariance among relatives as a function of the IBD relationships at each genetic marker locus. The IBD estimates for the IRAS Family Study pedigrees were determined using the LOKI computer program (28), in which IBD matrices were computed separately for the Hispanic and African-American families using ethnic-specific marker allele frequencies, combining the results for the linkage of the pooled family set. The residual heritability is partitioned into a component attributed to the QTL (linkage) and the residual additive genetic effects at that marker locus. The hypothesis of "no linkage" (heritability of the QTL equal to zero) was tested by comparing the likelihood of the restricted model with one in which the heritability due to the QTL was estimated. Twice the difference in ln(likelihood) of the two models is distributed asymptotically as a one-half, i.e., one-half mixture of a χ^2_1 and a point mass at zero (29). LOD scores are obtained by converting the ln(likelihood) values into $\log_{10}$ values. Maximum likelihood estimates of the model parameters were obtained, and the likelihood of the pedigree data were computed under each formulated model using the SOLAR (27) to obtain LOD scores and *P* values. Empirical LOD scores and *P* values were determined within SOLAR by simulation (lodadj procedure).

Genome scan microsatellite markers on chromosome 17 were examined for association using a GEE approach. Both allelic and genotypic associations were examined in which the analytic models accounted for familial correlation (family structure) using a sandwich estimator of the variance under exchangeable correlation assumption. Each model was adjusted for age, sex, clinic, and BMI. For each marker, $P < 0.01$ was used as significance for global association.

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ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health Grants HL060894 (to D.W.B.), HL060919 (to S.M.H.), HL060931 (to R.N.B.), HL060944 (to L.E.W.), HL061019 (to J.M.N.), and HL061210 (to M.F.S.), grants #M01-RR00865 and HL071141 (to C.C.H.), and the Cedars-Sinai Board of Governors Chair in Medical Genetics (to J.I.R.).

The authors gratefully acknowledge the dedication and work performed by the staff at the clinical sites in San Antonio, Texas, San Luis Valley, Colorado, and Los Angeles, California, laboratory staff at the University of Southern California, and the members of the Coordinating Center at Wake Forest University School of Medicine.

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