A Genome-Wide Scan for Loci Linked to Plasma Levels of Glucose and HbA_{1c} in a Community-Based Sample of Caucasian Pedigrees

The Framingham Offspring Study

James B. Meigs,[1] Carolien I. M. Panhuysen,[2] Richard H. Myers,[3] Peter W.F. Wilson,[4,5] and L. Adrienne Cupples[2]

Elevated blood glucose levels are the hallmark of type 2 diabetes as well as a powerful risk factor for development of the disease. We conducted a genome-wide search for diabetes-related genes, using measures of glycemia as quantitative traits in 330 pedigrees from the Framingham Heart Study. Of 3,799 attendees at the 5th Offspring Study exam cycle (1991–1995), 1,461, 1,251, and 771 men (49%) and women provided information on levels of 20-year mean fasting glucose, current fasting glucose, and HbA_{1c}, respectively, and 1,308 contributed genotype data (using 401 microsatellite markers with an average spacing of 10 cM). Levels of glycemic traits were adjusted for age, cigarette smoking, alcohol and estrogen use, physical activity, and BMI. We ranked standardized residuals from these models, created normalized deviates from the ranks, and used the variance component model implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines) to evaluate linkage to normalized deviates as quantitative traits. We found peak evidence for linkage to 20-year mean fasting glucose levels on chromosome 1 at ~247 cM from p-telomere (pter) (multipoint logarithm of odds [LOD] 2.33) and on chromosome 10 at ~86 cM from pter (multipoint LOD 2.07); to current fasting glucose levels on chromosome 1 at ~218 cM from pter (multipoint LOD 1.80) and on chromosome 10 at ~96 cM from pter (multipoint LOD 2.15); and to HbA_{1c} levels on chromosome 1 at ~187 cM (multipoint LOD 2.81). This analysis of unselected European Caucasian pedigrees suggests localization of quantitative trait loci influencing glucose homeostasis on chromosomes 1q and 10q. Findings at ~187–218 cM on chromosome 1 appear to replicate linkage reported in previous studies of other populations, pointing to this large chromosomal region as worthy of more detailed scrutiny in the search for type 2 diabetes susceptibility genes. *Diabetes* 51: 833–840, 2002

Type 2 diabetes is a common metabolic disorder defined by the presence of markedly elevated levels of plasma glucose (1). Subtle elevations in glucose levels commonly precede the development of type 2 diabetes and are a powerful predictor of future disease (2). Levels of glucose and HbA_{1c} (a time-integrated measure of antecedent glycemic status) (3,4) have continuous, graded relationships with risk for diabetes (5). These glycemic measures thus represent major quantitative traits associated with type 2 diabetes. Several lines of evidence suggest an underlying genetic basis for type 2 diabetes, including high concordance in monozygotic twins (6,7), greater prevalence rates among populations with greater admixture of Native American gene sources (8,9), and aggregation of type 2 diabetes in families (10–12). Linkage analyses have identified specific gene loci in a few families with maturity-onset diabetes of the young (13), but no specific gene or set of genes has been found for the common form of type 2 diabetes (14,15).

Incomplete understanding of the molecular basis for type 2 diabetes complicates the search for genes for this disease. Blood glucose levels are determined by a complex interplay between pancreatic β-cell function and sensitivity to insulin in hepatic and skeletal muscle cells (16). Obesity further modulates the phenotypic expression of diabetes (17). Genetic variation at several cellular sites is likely to influence regulation of glycemia, but linkage results from a wide array of promising candidate genes have been frustratingly difficult to replicate (18,19). In the setting of a heritable phenotype and an unknown complex genotype, whole-genome scanning represents one approach to the search for chromosomal regions potentially containing type 2 diabetes susceptibility genes. A number of whole-genome scan studies have been conducted in populations at high risk for diabetes—either high-prevalence populations, such as Pima Indians (where diabetes prevalence exceeds 40% by age 45 years) (20), or populations identified by type 2 diabetes probands—using the type 2 diabetes phenotype or levels of glucose as a

From the [1]General Internal Medicine and Clinical Epidemiology Units, General Medicine Division, Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts; the [2]Department of Epidemiology and Biostatistics, Boston University School of Public Health and School of Medicine, Boston, Massachusetts; the [3]Department of Neurology, Boston University School of Medicine, Boston, Massachusetts; the [4]Framingham Heart Study, Framingham, Massachusetts; and the [5]Boston University School of Medicine, Boston, Massachusetts.

Address correspondence and reprint requests to James B. Meigs, MD, MPH, General Medicine Division, Massachusetts General Hospital, 50 Staniford St. 9th Floor, Boston, MA 02114. E-mail: jmeigs@partners.org.

Received for publication 11 December 2000 and accepted in revised form 27 November 2001.

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

IBD, identical by descent; LOD, logarithm of odds; pter, p-telomere; QTL, quantitative trait locus.

quantitative trait. Suggestive linkage with the diabetes phenotype has been reported at several loci on chromosomes 1, 2, 4, 5, 8–12, 18, and 20 (21–38) and with glucose levels on chromosomes 1, 3, 6, 11, and 22 (39–41). In this report, we extend the genome-wide search for diabetes genes to unselected community-based pedigrees derived from the Framingham Offspring Study. These families are essentially all European Caucasian, with a cumulative incidence of type 2 diabetes of 14% by age 70 (12).

RESEARCH DESIGN AND METHODS

Subjects of the Framingham Offspring Study are the natural children and spouses of the children of the original Framingham Heart Study cohort families, aged 12–58 years at the first offspring examination in 1971–1975 (42). In 1995, 2,607 children in 1,643 pedigrees and 1,192 unrelated spouses completed 20 years of quadrennial follow-up at the fifth examination cycle (January 1991 through June 1995). These 3,799 subjects (1,792 men and 2,007 women) underwent a clinical and fasting laboratory examination, with written informed consent obtained before examination. We included in this analysis members of the largest 330 pedigrees (2,885 Framingham original cohort and offspring subjects), in whom 1,815 individuals were genotyped by the Marshfield Mammalian Genotyping Service. Genomic DNA was extracted from peripheral lymphocytes using a Qiagen Blood and Cell Culture DNA Maxi Kit. A genome-wide scan was carried out by the Marshfield Mammalian Genotyping Service. The set of 401 microsatellite markers (43) covers the genome at an average density of one marker every 10 cM and has an average heterozygosity of 0.77 (Screening Set version 8) (44). The screening set and genotyping protocols are available at the website of the Center for Medical Genetics, Marshfield Medical Research Foundation (http://research.marshfieldclinic.org/genetics/). Map distances were taken from Screening Set version 9 and the Marshfield "build your own map" facility. Marker locus D numbers were obtained from the "search for markers" facility; cryptic duplicates were replaced with Marshfield map markers. Familial relationships were checked using ASPEX (available at ftp://lahmed.stanford.edu/pub/aspex/doc/usage.html), which uses maximum likelihood estimation to test whether the genotyping data are consistent with the specified relationships, and adjustments were made accordingly. In addition, random genotyping errors were checked using the INFER procedure in PEDSYS (available at http://www.sfbr.org/sfbr/public/software/pedsys/pedsys.html). When we discovered a genotyping error and could not attribute it to a specific person in a nuclear family, all genotypes for that nuclear family were classified as missing. Using these procedures, 113 individuals with familial or genotyping errors were excluded, leaving 1,702 individuals in 330 pedigrees accurately genotyped, of whom 394 were original cohort members and 1,308 were offspring.

From among pedigrees with genotype data, 675 men and 706 women attended offspring exam 5. Of these 1,381 subjects, 1,370 had current fasting glucose measured, and 821 had HbA_{1c} measured. Over the five quadrennial examinations from the beginning of the Offspring Study, 1,461 offspring had three or more measures of fasting glucose for the calculation of the 20-year mean fasting glucose level. Glycemic trait data were not available from Original Cohort subjects; 394 of them (in 933 parent-offspring pairs) contributed information to improve estimates of identical by descent (IBD) sharing probabilities. Because of missing values in the independent parameters used in adjustment of glycemic traits, the final analysis included 1,461, 1,251, and 771 offspring contributing data on distributions of 20-year mean fasting glucose, current fasting glucose, and HbA_{1c}, respectively. Of 1,461 offspring in 330 pedigrees with mean glucose data, 1,267 also had genotype data; within pedigrees with mean glucose data, there were 1,662 sib-pairs (kinship coefficient 0.5), 206 avuncular and other second-degree relative pairs (kinship coefficient 0.25), 1,003 third-degree relative pairs (kinship coefficient 0.125), and 110 greater-than-third-degree relative pairs (kinship coefficient 0.0625–0.03125). Of 1,251 offspring in 328 pedigrees with fasting glucose data, 1,121 also had genotype data; within pedigrees with fasting glucose data, there were 1,236 sib-pairs, 149 second-degree relative pairs, 738 third-degree relative pairs, and 78 greater-than-third-degree relative pairs. Of 771 offspring in 265 pedigrees with HbA_{1c} data, 683 also had genotype data; within pedigrees with HbA_{1c} data, there were 672 sib-pairs, 66 second-degree relative pairs, 219 third-degree relative pairs, and 42 greater-than-third-degree relative pairs.

We classified offspring with diagnosed diabetes if they reported hypoglycemic drug therapy or had ≥2 fasting plasma glucose levels ≥7.8 mmol/l at any time during exams 1–5 (45). BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Smoking was assessed as the number of cigarettes smoked on average per day during the year before the examination. Physical activity was assessed as a weighted sum of the proportion of a typical day spent sleeping and performing sedentary, slight, moderate, or heavy physical activities (46). Alcohol use was categorized as usual consumption in ounces of alcohol per week. Estrogen use (either birth control or estrogen replacement therapy) was defined as present or absent. We measured plasma glucose with a hexokinase reagent kit (A-gent glucose test; Abbott, South Pasadena, CA). Glucose assays were run in duplicate, and the intra-assay coefficient of variation ranged from 2 to 3%, depending on the assayed glucose level. HbA_{1c} was measured by high-performance liquid chromatography after an overnight dialysis against normal saline to remove the labile fraction (47). The mean ± SD for this assay among normal glucose-tolerant subjects in this population was 5.22 ± 0.6%, and the inter- and intra-assay coefficients of variation were <2.5%. The assay was standardized against and comparable to the glycosylated hemoglobin assay used in the Diabetes Control and Complications Trial (48).

Descriptive statistics were computed for 20-year mean and current levels of fasting glucose, HbA_{1c}, and covariates using means ± SDs for continuous measurements and percentages for categorical measures. Linear regression models were used before linkage analyses to adjust for potentially confounding covariates, including age (including squared and cubic terms to allow for nonlinearity), smoking, alcohol use, physical activity, estrogen usage in women, and BMI (in kg/m^2). In men, these covariates accounted for 5–9% of the variability in glycemic measures, and in women they accounted for 12–16%. We also ran models calculating heritablities that did not include covariate adjustment or a term for BMI to demonstrate the effect of phenotype manipulation on residual polygenic variance. Models not including BMI were run based on the hypothesis that obesity may be in the causal pathway leading to type 2 diabetes; in general, adjustment for BMI strengthened linkage findings. Separate regression models were used for men and women; residuals were used for the linkage analyses including both men and women. In regression models, we included all subjects with available data (2,003 men and 2,130 women with 20-year mean fasting glucose; 1,786 men and 1,982 women with current fasting glucose; and 1,280 men and 1,452 women with HbA_{1c}). Because the glycemic quantitative trait distributions were highly skewed, we ranked the standardized residuals from regression analyses and created normalized deviates from the ranks, thereby forming normally distributed measures. Linkage analyses were performed on normalized deviates derived from adjusted levels of 20-year mean fasting glucose, current (exam 5) fasting glucose, and HbA_{1c}. We also repeated analyses excluding subjects with diagnosed diabetes (the 20-year mean fasting glucose, current fasting glucose, and HbA_{1c} numbers with diabetes were 171, 40, and 22, respectively). Linkage results including or excluding diagnosed diabetes were similar; only results of analyses including all subjects are shown.

We used the variance component model implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines) for quantitative traits to evaluate linkage to the 401 anonymous markers (49). This model makes no assumptions about the mode of transmission and uses all of the data from the pedigree without breaking it up into smaller subsets. The variance-component model partitions the variability of a multivariate normal trait into components for a quantitative trait locus (QTL), the residual polygenic component, and the random individual error. Linkage is evaluated by whether the variance component for the QTL is statistically significantly greater than zero. Comparison of likelihoods on a log base-10 scale, with the QTL variance set to zero and one, and with the QTL freely estimated, provides a logarithm of odds (LOD) score. Multipoint linkage analysis uses an extension of the Fulker-Cherny regression (50). We considered linkage results to represent genome-wide significance if the LOD score exceeded 3.0, and to represent suggestive linkage if the LOD score exceeded 1.5 (51). Using these thresholds with marker spacings of 10 cM, an LOD score of 3.0 would be expected to produce 5 false-positive linkage peaks in a whole-genome scan, for a LOD score of 1.5, ~10 false-positive results. These criteria were originally intended for analysis of discrete phenotypes in sib-pairs; their use may represent a more conservative approach when applied to analysis of quantitative traits in unselected pedigrees. We further evaluated the potential for obtaining false evidence for linkage by deriving the distribution of nominal LOD scores under the null hypothesis of no linkage by simulation using the SIMQTL script implemented in SOLAR. We simulated an unlinked marker using our pedigree structure and assigning a heritability of 0.40 for each glycemic trait. We assigned derived trait values for each trait to those subjects in our pedigrees who had the observed traits. We then performed two-point linkage analysis with the simulated unlinked marker to generate an expected LOD score under the null hypothesis. We repeated this procedure 1,000 times and estimated the proportion of samples that had LOD scores that were less than 1, 2, or 3. This strategy can be used to evaluate the potential proportion of false-positive results.

TABLE 1
Crude and adjusted trait heritabilities

	Mean glucose	Fasting glucose	HbA_{1c}
Subjects with trait (n)	1,461	1,251	771
Crude	0.50 ± 0.06	0.38 ± 0.07	0.39 ± 0.09
Age-adjusted	0.51 ± 0.06	0.42 ± 0.07	0.29 ± 0.08
Age- and BMI-adjusted	0.49 ± 0.06	0.36 ± 0.07	0.33 ± 0.09
Multivariate-adjusted	0.52 ± 0.06	0.38 ± 0.07	0.22 ± 0.08
Multivariate- and BMI-adjusted	0.51 ± 0.06	0.34 ± 0.07	0.27 ± 0.09

Data are heritability ± SE, unless otherwise indicated. Traits adjusted using sex-specific linear regression models; standardized residuals from models were ranked and heritabilities SE ± were obtained from variance components analysis of ranked deviates. Multivariate models included terms for age, age^2, age^4, cigarette smoking, alcohol consumption, physical activity, and estrogen replacement therapy (among women).

RESULTS

A total of 1,461 subjects provided information on 20-year mean fasting glucose levels; 1,251 subjects had current fasting glucose levels, and 771 subjects had HbA_{1c} levels. Of 1,461 subjects, 3% (40) had diagnosed diabetes, 51% were women, the mean age was 52 ± 10.2 years (range 26–82), and the mean BMI was 27.6 ± 5.1 kg/m^2 (14.4–53.8). The mean 20-year mean fasting glucose level was 5.4 ± 1.0 mmol/l (4.0–15.1), the mean current fasting glucose level was 5.6 ± 1.5 mmol/l (3.4–22.9), and the mean HbA_{1c} level was 5.29 ± 0.91% (4–11).

Normalized deviates derived from glycemic traits were used in all genetic analyses. Heritability estimates with progressive adjustment for confounding covariates and BMI are shown in Table 1. Phenotype manipulation had a minimal effect on heritability estimates for 20-year mean and current fasting glucose levels, but heritability declined by ~30% when comparing the fully adjusted HbA_{1c} trait with the crude HbA_{1c} trait.

For each glycemic trait, we used SOLAR to partition the total genetic variance into components due to a QTL and residual heritability, and we tested for linkage to 401 markers spaced at 10 cM average density throughout the genome. Although we did not find any chromosomal regions with genome-wide significant multipoint linkage (LOD >3.0), we found several regions with suggestive linkage (LOD >1.5). Maximum multipoint LOD scores for glycemic traits by chromosome and associated Marshfield marker identities are shown in Table 2. Detailed summaries of multipoint linkage analyses on chromosome 1 and 10 are shown in Figs. 1 and 2. Complete two-point and multipoint linkage results for each trait by chromosome are provided in an online appendix at http://diabetes.diabetesjournals.org.

TABLE 2
Maximum multipoint LOD score, map distance, and closest marker by trait per chromosome

	Mean glucose			Fasting glucose			HbA_{1c}		
Chromosome	Maximum multipoint LOD score	Map distance (cM)	Closest marker	Maximum multipoint LOD score	Map distance (cM)	Closest marker	Maximum multipoint LOD score	Map distance (cM)	Closest marker
1	2.33	247	D1S3462	1.80	218	D1S1678	2.81	187	D1S1589
2	1.02	101	GATA62B10	1.26	108	D2S2972	1.20	174	D2S1776
3	1.62	0	D3S4559	0.61	95	D3S4542	0.62	220	D3S2418
4	0.98	102	D4S1647	0.55	85	GATA168A08	0.13	0	D4S2366
5	0.62	185	D5S211	1.09	0	D5S2488	0.62	35	D5S2845
6	0.07	55	D6S2427	0.34	185	D6S1027	0.22	35	D6S1959
7	0.16	40	D7S1808	0.17	50	D7S817	0.70	80	D7S3046
8	0.11	70	D8S1136	1.09	115	GAAT1A4	0.33	70	D8S1110
9	0.31	45	D9S1121	0.02	0	D9S2169	1.49	60	D9S1118
10	2.07	86	GATA121A08	2.15	96	D10S1432	0.86	148	D10S1213
11	0.33	85	D11S2002	0.07	85	D11S2002	1.76	54	D11S1993
12	0.53	110	PAH	0.53	160	D12S1045	0.39	45	D12S1042
13	0.93	37	D13S325	0.32	50	D13S788	0.44	70	D13S892
14	0.49	125	D14S1426	0.80	125	D14S1426	0.09	115	D14S1434
15	0.67	95	D15S652	0.58	25	D15S165	0.83	90	D15S652
16	0.51	100	D16S516	0.39	100	D16S516	0.00	130	D16S2621
17	0.86	46	D17S2196	0.13	55	D17S1293	0.00	125	D17S928
18	0.01	65	D18S535	0.15	55	D18S877	0.04	80	D18S858
19	0.14	75	D19S246	0.31	75	D19S246	0.32	65	D19S178
20	0.00	95	D20S171	0.00	95	D20S171	0.93	8	GATA149E11
21	1.79	42	D21S2055	1.02	44	D21S2055	0.00	55	D21S1446
22	0.07	15	GCT10C10	0.66	0	D22S420	0.00	45	D22S445

Traits were adjusted using sex-specific linear regression models including terms for age, age^2, age^4, cigarette smoking, alcohol consumption, physical activity, estrogen replacement therapy (among women), and BMI. Standardized residuals from regression models were ranked, and quantitative trait analyses were conducted on these normalized deviates.

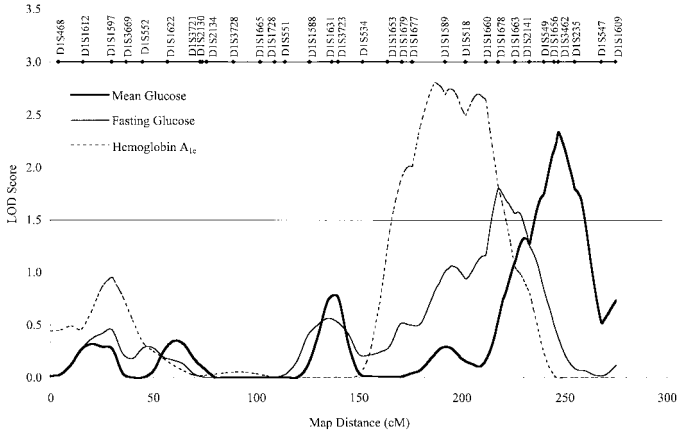

FIG. 1. Multipoint linkage analysis of 20-year mean fasting glucose, current fasting glucose, and HbA_{1c} levels. Data are multipoint LOD scores and marker identities on chromosome 1 for normalized deviates of glycemic traits adjusted for age; cigarette, alcohol, and estrogen use; physical activity; and BMI.

On chromosome 1q, we found peak evidence for linkage to levels of 20-year mean fasting glucose at ~247 cM from p-telomere (pter) (multipoint LOD 2.33, two-point LOD 3.79 at marker D1S3462). The 1–LOD unit CI around the region of peak linkage was 234–261 cM. Upstream at ~218 cM from pter, we found suggestive evidence for linkage to current fasting glucose levels (multipoint LOD 1.80, two-point LOD 2.42 at marker D1S1678; 1–LOD unit CI 186–241 cM). Further upstream at ~187 cM from pter, we found peak evidence of linkage to levels of HbA_{1c} (multipoint LOD 2.81, two-point LOD 1.20 at marker D1S1589; 1–LOD unit CI 170–218 cM). This linkage maximum included a subsidiary peak at 207 cM (multipoint LOD 2.70, two-point LOD 2.86 at marker D1S1660).

On chromosome 10q, we found peak evidence for linkage to levels of 20-year mean fasting glucose at ~86 cM from pter (multipoint LOD 2.07, two-point LOD 1.72 at marker GATA121A08; 1–LOD unit CI 65–99 cM). Nearby, we found evidence for linkage to current fasting glucose levels at ~96 cM from pter (multipoint LOD 2.15, two-point LOD 1.67 at marker D10S1432; 1–LOD unit CI 78–105 cM). Levels of HbA_{1c} did not show substantial evidence for linkage on chromosome 10, with only a weak peak at ~148 cM from pter (multipoint LOD 0.86, two-point LOD 0.98 at marker D10S1213).

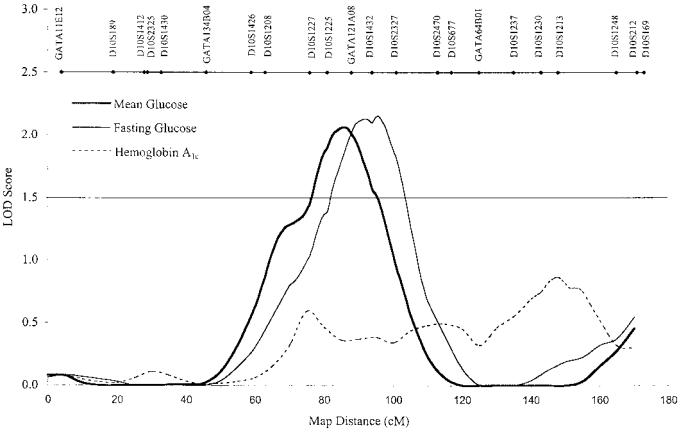

FIG. 2. Multipoint linkage analysis of 20-year mean fasting glucose, current fasting glucose, and HbA_{1c} levels. Data are multipoint LOD scores and marker identities on chromosome 10 for normalized deviates of glycemic traits adjusted for age; cigarette, alcohol, and estrogen use; physical activity; and BMI.

We also found suggestive evidence for linkage on several other chromosomes. On chromosome 3p, 20-year mean fasting glucose showed evidence for linkage at pter (multipoint LOD 1.62, two-point LOD 1.84, marker D3S4559). On chromosome 11p, levels of HbA_{1c} showed evidence for linkage at ~54 cM (multipoint LOD 1.76 at marker D11S1993, two-point LOD 1.53 at 33 cM, marker ATA34E08). On chromosome 21p, 20-year mean fasting glucose showed evidence for linkage at ~42 cM (multipoint LOD 1.79, two-point LOD 1.96 at marker D21S2055).

Simulation of linkage to an unlinked marker to a derived trait representing 20-year mean fasting glucose levels produced a median two-point LOD score of 0.0003; 2.7% of simulations produced an LOD score >1.0, 0.1% produced an LOD score >2.0, and 0% produced an LOD score >3.0. For current fasting glucose levels, simulation produced a median two-point LOD score of 0.0001; 2.0% of simulations produced an LOD score >1.0, 0% produced an LOD score >2.0 or >3.0. For HbA_{1c} levels, simulation produced a median two-point LOD score of 0.0; 2.0% of simulations produced an LOD score >1.0, 0.1% produced an LOD score >2.0, and 0% produced an LOD score >3.0.

DISCUSSION

Type 2 diabetes results from an interplay between specific environmental exposures and defects in insulin secretion and sensitivity. The physiological precursors of type 2 diabetes are most likely determined in part by multiple genes acting in concert to produce hyperglycemia (52,53). Results of our genome scan provide evidence for linkage to glycemic traits at several loci across the genome, consistent with the existence of multiple genetic determinants of hyperglycemia, each with modest effect. Most of our linkage loci are novel, not having been reported in other Caucasian pedigrees, and the probability values for most of our results are below the threshold for definite genome-wide significance. However, we did find two chromosomal regions with loci that appear to replicate findings from other studies.

Our data suggest that a QTL-influencing variation in levels of HbA_{1c} and current fasting plasma glucose maps to the long arm of chromosome 1, with peak evidence for linkage occurring at ~187–218 cM from pter. This is the same chromosomal region where Elbein et al. (21) reported significant nonparametric linkage (multipoint LOD 2.26 at 214 cM) to the type 2 diabetes phenotype in Caucasian families living in Utah. The Elbein et al. study included families of Northern European ancestry with type 2 diabetic sib-pairs, where diabetes onset was <65 years. The discrete type 2 diabetes trait was defined by medical therapy or by repeat screening with an oral glucose tolerance test, with subjects <45 years of age meeting criteria for impaired glucose tolerance also considered affected. Recently, Wiltshire et al. (37) also reported linkage in European diabetic (defined by treatment or hyperglycemia) sib-pairs to a locus at 206.0 cM on chromosome 1 (multipoint LOD 1.98, between D1S2799 and D1S452). Vionnet et al. (26) also reported linkage to markers upstream on 1q at 178 cM from pter (multipoint LOD 2.50)

in French lean type 2 diabetic sib-pairs. Steinle et al. (38) have also provided preliminary evidence for linkage on 1q in Old Order Amish families families. In Pima Indians, Hanson et al. (23) reported linkage on chromosome 1q at 171 cM ($P = 0.0004$) from pter when comparing concordant versus discordant sib-pairs, and at 192 cM ($P = 7.4 \times 10^{-6}$) from pter in sib-pairs with type 2 diabetes age-of-onset <25 years. Concordance between our findings and five other studies supports the hypothesis that a novel diabetes susceptibility locus resides in the region from ~187 to ~218 cM from pter (between markers D1S1598 and D1S1678) on chromosome 1q. Our findings and those of Elbein et al. and Wiltshire et al. focus especially on the region at 206–218 cM, between markers D1S1518 and D1S1678, at least in Caucasian subjects. This region contains several candidate genes potentially involved in regulation of glycemia, including APOA2 (apolipoprotein A2, potentially associated with glycemia through involvement in control of free fatty acid levels) (54), PKLR (pyruvate kinase, a key regulatory enzyme in glycolysis), and LMX1 (a regulator of insulin gene transcription) (55). We also found evidence for linkage to levels of 20-year mean fasting glucose at ~247 cM on chromosome 1. This region contains CAPN2, a calcium-activated neutral protease related to CAPN10, a candidate gene recently associated with type 2 diabetes in a Mexican-American population (56).

On chromosome 10, we found relatively strong linkage signals at ~86–96 cM for levels of both 20-year mean and current fasting glucose. Wiltshire et al. (37) also reported linkage in diabetic sib-pairs between 86.9 and 76.0 cM on chromosome 10q (LOD 1.99). This region contains at least one interesting candidate gene, HK1 (hexokinase 1), the gene encoding a key enzyme in the initial step of glucose metabolism (57). We also found a weak, potentially false-positive peak for levels of HbA_{1c} at ~148 cM from pter on chromosome 10. In this chromosomal region, Duggirala et al. (24) previously reported significant linkage to the discrete type 2 diabetes trait (LOD 2.88) and the quantitative trait for diabetes age of onset (LOD 3.75) in Mexican-American families. Concordance between our results and those of Wiltshire et al. and Duggirala et al. strengthen the claim for the presence of a diabetes susceptibility locus on the long arm of chromosome 10. In particular, the region between 76 and 94 cM (markers D10S1227 and D10S1432) needs to be examined more closely using dense-marker mapping to narrow the region of greatest linkage.

Although we found suggestive linkage with QTLs for glycemic traits at loci on chromosomes 3p, 11p, and 21p, peak evidence for linkage occurred in regions distant from previously reported loci associated with other measures of glucose intolerance. On chromosome 3, Watanabe et al. (41) found evidence of linkage (LOD = 2.26) to levels of C-peptide at 58 cM in diabetic subjects and to levels of fasting glucose at 40 cM (LOD = 2.22) in nondiabetic subjects. On chromosome 11, Stern et al. (39) found evidence of linkage to 2-h postchallenge levels of glucose at 14 cM (LOD = 2.77) and 23–35 cM (LOD = 3.37); Hanson et al. (23) reported linkage to the diabetes phenotype at 137 cM (LOD = 1.87). In addition, we did not replicate previous linkages with type 2 diabetes or glycemic traits reported on chromosomes 2 (22,25), 4 (28), 6 (39), 9 (36), 12 (33,34,58), 14 (41), 18 (29), 19 (41), 20 (28,30–32,35,36), or 22 (40). Differences across studies are likely attributable to variation in relatively weak linkage signals from individual diabetes susceptibility genes combined with population-specific variation in major genetic determinants of type 2 diabetes. Statistical methodologies and phenotype definitions also differ across studies. Our analysis has two important, unique differences from these prior studies. First, we analyzed an unselected sample derived from a community base at moderate risk for type 2 diabetes. Second, we used normalized deviates (adjusted for important confounders in the association between physiological determinants of glycemia and phenotypic expression of type 2 diabetes) as quantitative traits. Although this approach "averages" the underlying trait to some degree and may have obscured some elements of individual variation, the method does produce a quantitative trait relatively free of confounding influences derived directly from the phenotype for each individual. Most importantly, ranking the deviates produced a perfectly normal distribution in the trait, where lack of skewness and kurtosis minimized the possibility that extreme values produced spurious linkage signals that introduce type I error (59). The relatively broad shape of the linkage peaks on chromosomes 1 and 10 adds further support in favor of these being true-positive rather than false-positive linkage loci (60). Our simulation results also indicate that our findings are unlikely to be spurious false-positive peaks: given our observed pedigree structure, for each trait there was only a 1 in 1,000 (or less) probability of finding a peak with a 2-point LOD of ≥2.0.

One potential limitation of our analysis is that extensive adjustment of traits for confounding covariates may potentially overestimate apparent underlying genetic components. In particular, adjustment for BMI has the potential to weaken linkage signals, especially since obesity may be in the physiological causal pathway to type 2 diabetes (61,62) and since there may be important pleiotropy in the genetic underpinnings of diabetes and obesity (23,63). On the other hand, populations at risk for diabetes are an admixture of phenocopies and subjects at increased genetic risk for diabetes on the basis of obesity; failure to account for population variation in BMI could also increase the probability of type II error. In any case, for levels of 20-year mean and current fasting glucose, varying degrees of covariate adjustment had little substantive influence on their heritability estimates. Heritability of the HbA_{1c} level was more sensitive to covariate adjustment, suggesting that smoking, physical activity, age, or other nongenetic exposures may exert fairly strong effects on its phenotypic expression (64). Furthermore, factors that influence the average life span of erythrocytes are likely to interact with levels of glycemia on expression of HbA_{1c}. For example, persons heterozygous for mutations in the pyruvate kinase gene may have a mild hemolytic anemia (65). Interestingly, pyruvate kinase is also a key enzyme in glycolysis, and its gene, PKLR, maps to ~164–167 cM on chromosome 1q, near the linkage peak we observed for HbA_{1c}. Thus, although HbA_{1c} is an excellent biomarker of average glycemia and diabetes control, its value as a diabetes quantitative trait for genetic analysis is somewhat unclear.

Another finding deserving comment is that in several instances we found multipoint LOD scores to be somewhat lower than the associated 2-point LOD scores. For complex traits with substantial locus heterogeneity, this finding, although seemingly counterintuitive, is not unexpected. Multipoint LOD scores are calculated from multipoint IBD estimates derived from a regression model that smoothes the 2-point IBD values in that region. When there are several elevated 2-point LOD scores in a region (as might be expected with a simple Mendelian trait), the multipoint LOD scores generally will be at least as high as the 2-point scores. However, when there is only a single high 2-point LOD score in a region along with lower LOD scores at surrounding marker loci, multipoint LOD scores will be lower than the 2-point score at that locus.

In conclusion, our results suggest that genes influencing glucose homeostasis and potentially predisposing to type 2 diabetes may be located on chromosomes 1q and 10q. We found the strongest evidence for linkage with glycemic traits at ~207–218 cM from pter on chromosome 1, a region thought to contain a diabetes susceptibility gene, based on data accumulating from several other studies. On chromosome 10, co-localization of linkage peaks for 20-year mean and current fasting plasma glucose levels at ~86–96 cM from pter (in the region of the candidate gene HK1), replicating findings from another recent study of European subjects. Replication of results across studies suggests that linkage peaks within these specific regions on chromosomes 1, and possibly 10, are true-positive findings. Further work is required to test associations of candidate genes in these regions with occurrence of type 2 diabetes, as well as to map other likely diabetes genes within these chromosomal regions.

ACKNOWLEDGMENTS

Work of the National Heart, Lung and Blood Institute (NHBLI) Mammalian Genotyping Service was supported in part by a grant from the National Institutes of Health (NIH)/NHLBI (PO1-HL41484). J.B.M. was supported by a clinical research grant from the American Diabetes Association and a junior faculty development award from SmithKline Beecham. P.W.F.W. was supported in part by a grant from Roche Laboratories. L.A.C. and the NHLBI's Framingham Heart Study are supported by a contract from the NIH (NIH/NHLBI contract NO1-HC-38083).

We thank David M. Nathan, M.D., and Daniel E. Singer, M.D., for help with collection and analysis of levels of HbA_{1c}; Alan Herbert, M.D. Ph.D., for helpful comments on an earlier version of this manuscript; James Weber, Ph.D., and the NHLBI Mammalian Genotyping Service for conducting the genome-wide scan; and Serkalem Demissie for assistance with stimulations.

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