A Genome-Wide Linkage Scan of Insulin Level–Derived Traits

The Amish Family Diabetes Study

Wen-Chi Hsueh,[1] Kristi D. Silver,[2] Toni I. Pollin,[2] Callum J. Bell,[3] Jeffrey R. O'Connell,[2] Braxton D. Mitchell,[2] and Alan R. Shuldiner[2,4]

OBJECTIVE—Serum insulin levels are altered in insulin resistance and insulin deficiency, states that are associated with the development of type 2 diabetes. The goal of our study was to identify chromosomal regions that are likely to harbor genetic determinants of these traits.

RESEARCH DESIGN AND METHODS—We conducted a series of genetic analyses, including genome-wide and fine-mapping linkage studies, based on insulin levels measured during an oral glucose tolerance test (OGTT) in 552 nondiabetic participants in the Amish Family Diabetes Study. Indices of insulin secretion included the insulinogenic index and insulin at 30 min postglucose load (insulin 30), while indices of insulin resistance included homeostasis model assessment of insulin resistance (HOMA-IR) and fasting insulin. Insulin area under the curve, a measure of both insulin secretion and insulin resistance, was also examined.

RESULTS—All traits were modestly heritable, with heritability estimates ranging from 0.1 to 0.4 (all $P < 0.05$). There was significant genetic correlation between fasting insulin and HOMA-IR ($\rho_G > 0.86$, $P < 0.05$), as well as insulin 30 and insulinogenic index ($\rho_G = 0.81$, $P < 0.0001$), suggesting that common genes influence variation in these pairs of traits. Suggestive linkage signals in the genome scan were to insulin 30 on chromosome 15q23 (logarithm of odds [LOD] 2.53, $P = 0.00032$) and to insulinogenic index on chromosome 2p13 (LOD 2.51, $P = 0.00034$). Fine-mapping study further refined our signal for insulin 30 on chromosome 15 (LOD 2.38 at 68 cM).

CONCLUSIONS—These results suggest that there may be different genes influencing variation in OGTT measures of insulin secretion and insulin resistance. ***Diabetes*** **56:2643–2648, 2007**

Type 2 diabetes is a classic example of a complex disease that results from the interaction of multiple genetic and environmental factors. One strategy to identify the genes regulating the type 2 diabetes phenotype is to focus on related subclinical (intermediate) phenotypes, which are likely to be less genetically complex and involve fewer alleles. The pathophysiology of type 2 diabetes involves defects in insulin sensitivity and/or insulin secretion thus making these measures excellent intermediate traits to study in order to dissect the genetic underpinnings of type 2 diabetes. Circulating levels of plasma insulin, measured in either the fasting state or in response to a glucose load, vary considerably in nondiabetic individuals, with higher levels predicting future development of diabetes. Several indices of insulin secretion and insulin resistance can be derived from insulin and glucose levels measured at different time points during an oral glucose tolerance test (OGTT). OGTT-derived measures of insulin secretion include the insulinogenic index and insulin at 30 min following a 75-g oral glucose load (insulin 30), while measures of insulin resistance include fasting insulin and the homeostasis model assessment of insulin resistance (HOMA-IR). Insulin area under the curve (insulin AUC) during the OGTT reflects elements of both insulin secretion and insulin resistance.

Several studies have investigated the genetic epidemiology of fasting insulin levels and OGTT-derived indices (1,2). Genome-wide scans have identified several chromosomal regions linked to these traits (3–13), but few have been replicated. The goal of this study was to characterize the genetic epidemiology of five insulin-related traits, including fasting insulin, insulin 30, insulinogenic index, HOMA-IR, and insulin AUC. We first estimated their heritability, then assessed whether and to what degree these traits may share common genetic influence, and performed genome-wide and fine-mapping linkage analyses of these traits. We found that these insulin traits were significantly heritable and that there may be different genetic influences underlying these OGTT-derived measures of insulin secretion and resistance. Furthermore, our genome scan provides evidence of linkage on chromosomes 2p and 15q to measures of insulin secretion.

Clinical characteristics of 552 nondiabetic subjects are shown in Table 1. The overall prevalence of impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG) was 22% in this sample. Insulin resistance–related measures in those with IGT/IFG were significantly higher ($P < 0.001$) than in euglycemic individuals, whereas

From the [1]Department of Medicine, School of Medicine, University of California, San Francisco, California; the [2]Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland; [3]Axys Pharmaceuticals, La Jolla, California; and the [4]Geriatrics Research and Education Clinical Center, Baltimore Veterans Administration Medical Center, Baltimore, Maryland.

Address correspondence and reprint requests to Wen-Chi Hsueh, MPH, PhD, 2200 Post St., C433, San Francisco, CA 94143-1640. E-mail: wen-chi.hsueh@ucsf.edu.

Received for publication 24 July 2006 and accepted in revised form 17 July 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 23 July 2007. DOI: 10.2337/db06-1023.

C.J.B. is currently affiliated with EmerGen, Inc., Salt Lake City, Utah.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1023.

AUC, area under the curve; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; STR, short tandem repeat.

TABLE 1
Plasma levels of traits in nondiabetic study subjects by sex*

Trait	Male	Female	$h^2 \pm$ SE†
n	262	290	
Age (years)	46.0 ± 14.5	44.1 ± 14.6	—
BMI (kg/m^2)	26.3 ± 3.6	28.0 ± 5.5	0.40 ± 0.08‡
Fasting glucose (mmol/l)	5.09 ± 0.49	5.02 ± 0.47	0.48 ± 0.09
Glucose 30 (mmol/l)	8.21 ± 1.76	8.21 ± 1.52	0.31 ± 0.08
Fasting insulin (mU/l)	10.3 ± 3.8	11.0 ± 6.4	0.12 ± 0.07§
Insulin 30 (mU/l)	49.1 ± 29.7	54.6 ± 39.3	0.19 ± 0.10§
Insulinogenic index (units/g)	0.75 ± 0.72	0.87 ± 0.80	0.36 ± 0.11‡
HOMA-IR (mU per mmol/l^2)	42.2 ± 17.1	43.7 ± 20.1	0.13 ± 0.06§
Insulin AUC (mU · l^{-1} · h^{-1})	99.9 ± 54.8	145.1 ± 87.8	0.28 ± 0.10§

Data are means ± SD. *See ref. 25 for additional characteristics of the Amish Family Diabetes Study. Due to missing data points, sample sizes ranged from 552 for fasting insulin to 486 for insulin AUC. †Heritability ± SE, adjusted for effects of BMI, sex-specific age, and age^2. ‡$P < 0.001$; §$P < 0.05$.

insulin secretion–related measures were similar between the two groups (data not shown).

Heritability (h^2) estimates for insulin-related traits ranged from 0.12 to 0.36 (all $P < 0.05$) (Table 1). We next examined the extent to which these traits share common genetic influences in order to assist our interpretation of subsequent linkage analyses. As shown in Table 2, high genetic correlations were observed between fasting insulin and HOMA-IR, suggesting that these two measures share very substantial genetic components (estimated $\rho_G = 0.86$, $P < 0.05$). These findings are not unexpected since HOMA-IR is derived from the fasting insulin. Insulin 30 had a strong genetic correlation with insulinogenic index ($\rho_G = 0.81$, $P < 0.001$). Insulin AUC was more genetically correlated with insulin 30 than with any other trait ($\rho_G = 0.82$, $P < 0.001$). There was no significant genetic correlation between fasting insulin and insulin 30 or the insulinogenic index.

Our genome-wide linkage analysis identified two chromosomal regions with suggestive evidence for linkage (defined as $P < 0.001$ or logarithm of odds [LOD] >2.07) to insulin secretion traits. The first region was for insulin 30 (LOD 2.53, $P = 0.00032$), occurring at 73 cM on chromosome 15q23 (nearest marker D15S131) (Fig. 1*A*), which was also supported by the two-point analysis of D15S131 (LOD 2.82, $P = 0.00016$). The second region was for insulinogenic index at 121 cM on chromosome 2p13 (LOD 2.51, $P = 0.00034$; nearest marker D2S139) (Fig. 1*B*), as supported by two-point analysis of D2S139 (LOD 1.45, $P = 0.0049$). Similar results were obtained when the analysis was performed without adjustment for BMI. Nine other chromosomal regions (on chromosomes 1q21, 2q21-q22, 6p24-p23, 7q11-q21, 7q31, 10q11, 11q21-q23, 12p13-p12, and 19p13) showed linkage signals to one or more insulin traits with LOD ≥1.18 ($P < 0.01$) (Table 3). Bivariate linkage analysis for the two pairs of highly correlated insulin secretion traits was conducted. The maximum LOD score for (insulin 30 + the insulinogenic index) was 2.21 at 69 cM from the p-ter of chromosome 15. Insulin 30 + insulin AUC showed a lower LOD score (1.58) at 73 cM, also on chromosome 15. These results suggest that the genes in this region may contribute more to the insulin 30 phenotype than to the other two traits. The complete genome scan results can be viewed in the online appendix (available at http://dx.doi.org/10.2337/db06-1023).

To follow up on the linkage signal to insulin 30 on chromosome 15q, 14 additional short tandem repeat (STR) markers were genotyped to increase the information con-

TABLE 2
Correlation coefficients* among insulin traits†

	Fasting insulin	HOMA-IR	Insulin 30	Insulinogenic index
HOMA-IR				
ρ_P	0.92 ± 0.00			
ρ_G	0.86 ± 0.08‡			
ρ_E	0.93 ± 0.004			
Insulin 30				
ρ_P	0.52 ± 0.03	0.50 ± 0.04		
ρ_G	0.18 ± 0.34	0.15 ± 0.34		
ρ_E	0.59 ± 0.05	0.57 ± 0.05		
Insulinogenic index				
ρ_P	0.34 ± 0.04	0.28 ± 0.04	0.75 ± 0.02	
ρ_G	0.41 ± 0.27	0.16 ± 0.29	0.81 ± 0.08§	
ρ_E	0.34 ± 0.08	0.34 ± 0.08	0.75 ± 0.04	
Insulin AUC				
ρ_P	0.62 ± 0.03	0.59 ± 0.03	0.75 ± 0.02	0.46 ± 0.04
ρ_G	0.52 ± 0.23	0.32 ± 0.29	0.82 ± 0.10§	0.38 ± 0.21
ρ_E	0.66 ± 0.05	0.65 ± 0.05	0.74 ± 0.04	0.50 ± 0.08

*ρ_P, phenotypic correlation; ρ_G, genetic correlation; ρ_E, random environmental correlation. †All ρ_P and ρ_E are with $P < 0.001$. ‡$P < 0.05$; §$P < 0.001$.

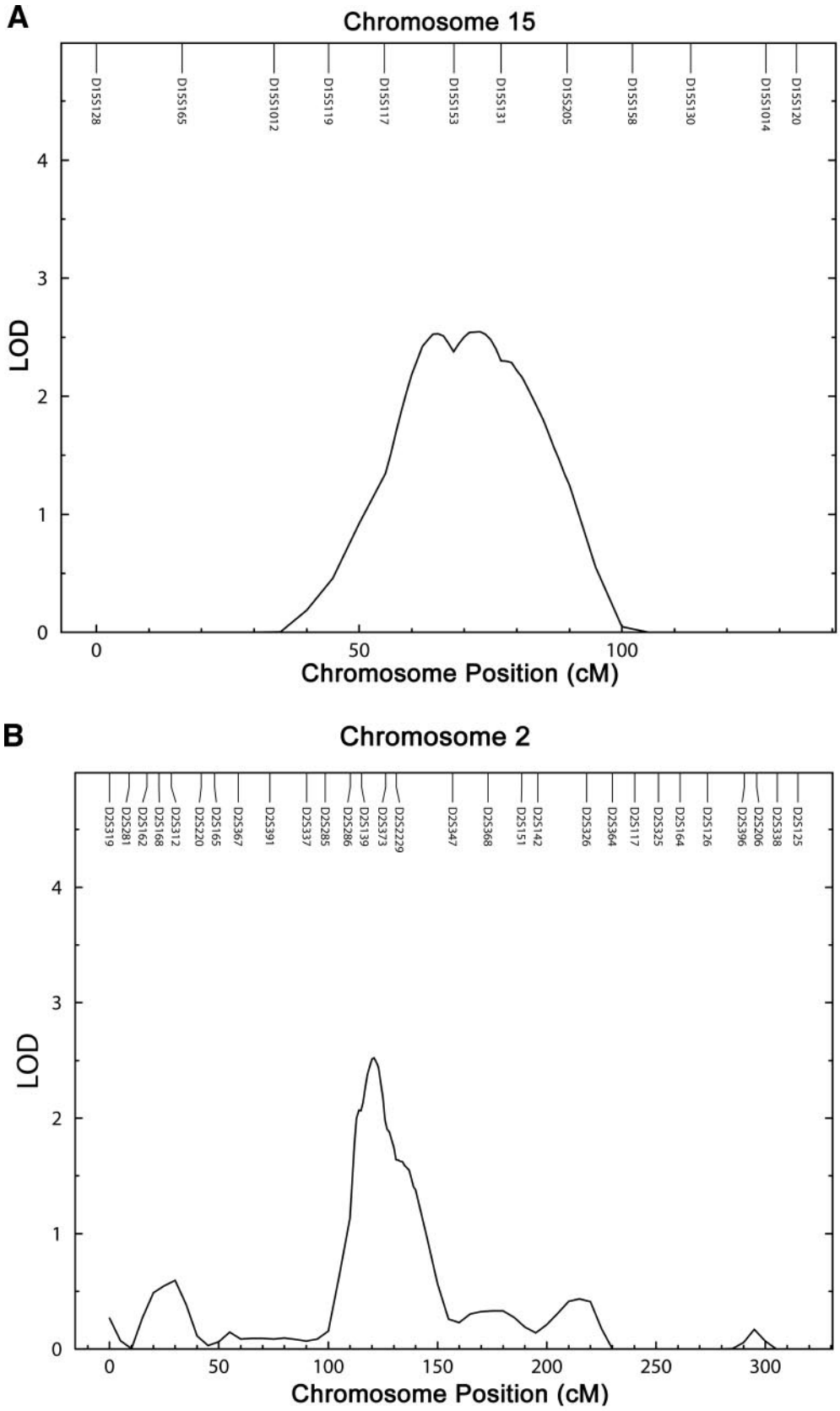

FIG. 1. *A*: Multipoint linkage analysis results on chromosome 15 for insulin at 30 min. *B*: Multipoint linkage analysis results on chromosome 2 for the insulinogenic index.

TABLE 3
LOD scores >1.18 ($P < 0.01$) for each insulin-related trait from multipoint linkage analyses

Chromosome	Trait	Position (cM)	Nearest STR marker	LOD score
1q21	HOMA-IR	145	D1S420	1.19
2p13	Insulinogenic index	121	D2S373	2.51
2q21-q22	Fasting insulin	315	D2S125	1.58
6p24-p23	Insulinogenic index	10	D6S344	1.18
7q11-q21	HOMA-IR	105	D7S669	1.88
7q31	Fasting insulin	125	D7S657	1.36
	Insulin AUC	142	D7S657	1.54
10q11	Insulinogenic index	75	D10S220	1.19
11q21-q23	Insulin 30	115	D11S1358	1.29
12p13-p12	Fasting insulin	35	D12S364	1.27
15q23	Insulin 30	73	D15S131	2.53
19p13	Fasting insulin	10	D19S216	1.32

tent in the region. These markers reduced the marker density from 11.1 to 3.2 cM and further refined the linkage peak to near D15S153 (at 68 cM, LOD 2.38), ~9 cM closer to the centromere compared with the original genome scan results, which was well within the 1-LOD interval.

Insulin secretion and insulin sensitivity are important determinants of glucose homeostasis and diabetes. The current gold standard for quantifying insulin secretion is the acute insulin response to intravenous glucose (AIR_g) test and, for insulin sensitivity, the hyperinsulinemic-euglycemic clamp. Since it is not practical to obtain these labor-intensive and costly phenotypes in large numbers of subjects for genetic studies, many have used surrogate measures of insulin secretion and insulin sensitivity. Previous studies have shown that the correlation between insulin-related indices from OGTT investigated in this study and those measured in the clamp were moderate ($r = 0.3–0.7$) but significant (14,15). In contrast, Bergman et al. (16) showed that in African and Hispanic Americans, the ρ_G between fasting insulin and HOMA-IR was high (0.96), while the ρ_G between fasting insulin and insulin sensitivity (S_i) derived from an intravenous glucose tolerance test and that between HOMA-IR and S_i were modest ($\rho_G = -0.46$ and -0.48, respectively). The authors interpreted these findings to mean that compared with S_i, fasting insulin and HOMA-IR are not good proxy measurement of insulin resistance. However, another interpretation is that there may be differential genetic influence on insulin resistance among different populations or that HOMA-IR and S_i may be measuring different aspects of insulin resistance. Indeed, variation in the relative amount of hepatic versus muscular insulin resistance has been demonstrated in individuals with type 2 diabetes, and fasting glucose and insulin mark hepatic insulin resistance, while S_i may be a better marker of muscular insulin resistance (1,2).

Our systematic genetic analyses of plasma insulin levels during a 3-h OGTT and indices derived from these measures provide insights that are likely to be useful in the search for genetic influences on insulin secretion and sensitivity. We demonstrate modest levels of heritability for a number of insulin secretion–related traits that have not been previously reported, such as insulin AUC, insulinogenic index, and insulin 30. The level of heritability for fasting insulin levels in the Amish was relatively low ($h^2 = 0.13$) compared with values reported in Caucasians (0.37–0.47) (17,18), African Americans (0.28) (18), Mexican Americans (0.38–0.53) (4,19), Pima Indians (0.26) (15), and Asians (0.43) (20), although not much different from that estimated in African Americans and Hispanics from the Insulin Resistance and Atherosclerosis Study ($h^2 = 0.08–0.17$) (16,21).

As expected, based on the fact that HOMA-IR is derived from fasting insulin, both of these traits are phenotypically and genetically highly correlated. These two traits may reflect relatively more of the insulin-resistance phenotype. HOMA-IR is commonly used in epidemiological studies as a proxy measurement of insulin resistance, yet it did not appear to be more informative compared with fasting insulin in our genetic analysis. This is likely due to the fact that fasting insulin is a primary component of HOMA-IR calculation, particularly in nondiabetic subjects for whom there is less variation in glucose levels. Our results suggest that a simpler measurement (fasting insulin) may serve as well as a more complicated composite measurement (HOMA-IR) for genetic studies of insulin resistance. On the other hand, insulin 30 shared a significant genetic component with the insulinogenic index—both measures are thought to be more related to insulin secretion. Importantly, there was no significant genetic correlation between fasting insulin and insulin 30, suggesting that these two traits are likely to have different genetic influences. These findings are consistent with the biological concept that defects in insulin sensitivity and insulin secretion are genetically distinct.

From our genome-wide linkage analysis, we identified two chromosomal regions, 15q23 for insulin 30 and 2p13 for insulinogenic index, with suggestive evidence of linkage. For the linkage signal for insulin 30 on chromosome 15q, the 1-LOD support interval is a 31.4-cM region defined by markers D15S117 and D15S158. Within this interval, there are 302 genes (263 named, 59 predicted [NCBI Build 35.1]). Several genes within our region of linkage on 15q23 may potentially be associated with β-cell development and function. Some of the candidate genes in this region are involved with hormone secretion (*SCAMP2* and *SCAMP5*) (22,23), others are growth factors (*NRG4*) (24), while others such as *ISL2* are homologous to proteins known to be involved in β-cell development (*ISL1*) (25). For the linkage signal for insulinogenic index on chromosome 2p, the 1-LOD support interval is a 26-cM region defined by markers D2S139 and D2S347. Within this interval, there are 194 genes (167 named, 27 predicted). A number of candidate genes for β-cell development or function lie within the 1-LOD region, including two genes encoding secretory vesicle associated membrane proteins *VAMP5* and *VAMP8*.

It is somewhat surprising that there was little overlap of

linkage signals for insulin 30 and insulinogenic index on chromosomes 2 and 15 given that genetic correlation was high. However, these traits were not perfectly correlated and thus may not have an identical genetic basis. Furthermore, gene-by-gene or gene-by-environment interactions, which were not accounted for in estimates of genetic correlation, may contribute to the lack of significant overlapping linkage signals in these regions.

Thus far, genome-wide linkage studies of diabetes-related traits, including insulin traits, have been reported in several ethnic groups (3–9). Studies conducted in Japanese and Mexican Americans have also revealed linkage to type 2 diabetes on chromosome 15q. In a Japanese study (8), a suggestive linkage signal to type 2 diabetes was observed on 15q13-q21 (maximum LOD score = 2.19), whose 1-LOD region overlaps with the 1-LOD region for the linkage to insulin 30 in the Amish. A small linkage signal to type 2 diabetes (two-point maximum LOD score for D15S119 = 1.50) was also observed in the same region in a study of Mexican Americans (9). On the other hand, evidence for replication of our linkage on chromosome 2p13 to insulinogenic index (LOD 2.51 or empirical P = 0.00034, at 121 cM) is more limited. A meta-linkage analysis utilizing information from four ethnic groups of the National Heart, Lung, and Blood Institute Family Blood Pressure Program (3) reported suggestive linkage signals to both fasting insulin and HOMA-IR (LOD 2.3–2.6 or empirical P = 0.03–0.06, at ~113–117 cM on the Amish map).

We also observed LOD scores ≥1.18 (corresponding to an empirical $P < 0.01$) on nine other chromosomal regions. Several of these regions of linkage have also been reported in the literature. In a recent study, Freedman et al. (6) observed suggestive linkage to both fasting insulin and HOMA-IR in African Americans at the same location on chromosome 19p (LOD 2.3, near D19S1034, at ~10 cM on the Amish map) where we observed a modest linkage signal to fasting insulin (LOD 1.3 at 10 cM). We did not observe evidence for linkage to regions on chromosomes 3p (4), 19q (5), or 20p (6), which were previously reported to harbor loci for insulin traits we examined.

In summary, we observed evidence for linkage of insulin traits to regions of chromosome 2p13 and 15q23—both regions with a number of β-cell candidate genes. Further examination of these regions through linkage disequilibrium mapping and positional candidate gene analysis will be necessary to identify the genes and their functional variants, which should be relevant not only to their influences on insulin levels but also to susceptibility to type 2 diabetes.

RESEARCH DESIGN AND METHODS

Our study was based on 691 members of the Amish Family Diabetes Study. Details of subject recruitment have been previously reported (26). The study protocol was approved by the institutional review board at the University of Maryland, Baltimore, and informed consent was obtained from each participant.

Phenotypes. After an overnight fast, a standard 3-h OGTT with blood sampling every 30 min was administered to subjects without a prior history of diabetes. Plasma glucose and insulin concentrations were assayed with standard protocols. Fasting glucose levels ranged from 3.81 to 6.83 mmol/l in this population. Total insulin AUC during the 3-h OGTT was calculated using the trapezoid method. HOMA-IR index was calculated as [fasting insulin (mU/l) × fasting glucose (mmol/l)]/22.5. The insulinogenic index was calculated as [(insulin 30 − fasting insulin)/(glucose 30 − fasting glucose)]. Criteria for the diagnosis of type 2 diabetes, IGT, and IFG were adapted from American Diabetes Association recommendations. As the development and treatment of type 2 diabetes can significantly alter insulin levels, only nondiabetic subjects were included in the analysis (n = 552).

Genotypes. We typed 357 STR markers on 22 autosomes using DNA from leukocytes. These markers were from the ABI Prism Linkage Mapping set (Perkin-Elmer). Overall genotyping rates across all STRs were 96.3 ± 2.4% complete. The marker order and sex-averaged intermarker distances (mean 9.7 cM) were estimated from our data using CRI-MAP (27). The mean marker heterozygosity was 0.75 (range 0.33–0.91). Based on initial linkage analysis results, 14 additional STR markers were genotyped on chromosome 15 (between 14 and 101 cM) for a fine-mapping study.

Statistical analysis. Values for traits with a significantly skewed distribution were transformed by their natural logarithm, and extreme outliers (value deviating from the mean by >3 SD) were excluded from analysis (n = 0–4, depending on the trait). Furthermore, to reduce the computational complexity, we divided the single large pedigree into 27 smaller pedigrees (n = 3–118) for analysis. Results from all analyses were adjusted for sex-specific age, age^2, and BMI.

All analyses were conducted using a pedigree-based variance components method. We first estimated heritability then genetic correlation. Bivariate modeling was used to partition the phenotypic correlation (ρ_P) between a given pair of quantitative traits into their additive genetic (ρ_G) (i.e., genetic correlation) and random environmental (ρ_E) components (28). For linkage analyses, the effect of a quantitative trait locus was estimated by modeling the covariance in a trait between individuals to be a function of the probability that they inherited both alleles at the marker locus from a common ancestor. Both two-point and multipoint analyses were performed, and statistical significance was evaluated by likelihood ratio tests using the SOLAR program (29). Multipoint identity-by-descent matrices were computed using the Kosambi function in the LOKI program (30). As the variance components methods can be susceptible to significant violations of the multivariate normality assumption, we used simulations to estimate the empirical probability of obtaining false evidence for linkage. We derived the distribution of nominal LOD scores under the null hypothesis of no linkage by simulating 10,000 unlinked markers, dropping them through the pedigrees, and conducting linkage analysis with each of the 10,000 markers for each of the insulin traits. The probability of obtaining a false-positive result was defined as the proportion of replicates for which we obtained a specified LOD score or higher. The P values obtained from the simulation study were then back-converted into LOD scores by first converting them into corresponding χ^2 values and then by dividing the χ^2 values by (2 × ln10). All LOD scores from quatitative trait locus analyses presented in this report were obtained from this simulation.

ACKNOWLEDGMENTS

This study was supported in part by a research grant from GlaxoWellcome, Inc. and Axys Pharmaceuticals; National Institutes of Health grants R01 DK54361, K24 DK02673, U01 DK58026, R01 AG023692, R01 DK068495, and K01 AG022782; and the American Diabetes Association. Funding and support was also provided by the University of Maryland General Clinical Research Center (Grant M01 RR 16500), General Clinical Research Centers Program, National Center for Research Resources, and the Baltimore Veterans Administration Geriatric Research and Education Clinical Center.

We thank the Amish Research Clinic Staff for their energetic efforts in study subject recruitment and characterization, Drs. Alejandro Schäffer and Richa Agarwala for assistance in pedigree construction, and Dr. Pamela St. Jean for helpful comments on the manuscript. This study would not have been possible without the outstanding cooperation of the Amish community.

REFERENCES

1. Mercado MM, McLenithan JC, Silver KD, Shuldiner AR: Genetics of insulin resistance. *Curr Diab Rep* 2:83–95, 2002
2. Stumvoll M, Fritsche A, Haring HU: Clinical characterization of insulin secretion as the basis for genetic analyses. *Diabetes* 51 (Suppl. 1):S122–S129, 2002
3. An P, Freedman BI, Hanis CL, Chen YD, Weder AB, Schork NJ, Boerwinkle E, Province MA, Hsiung CA, Wu X, Quertermous T, Rao DC: Genome-wide linkage scans for fasting glucose, insulin, and insulin resistance in the National Heart, Lung, and Blood Institute Family Blood Pressure Program:

evidence of linkages to chromosome 7q36 and 19q13 from meta-analysis. *Diabetes* 54:909–914, 2005
4. Mitchell BD, Cole SA, Hsueh WC, Comuzzie AG, Blangero J, MacCluer JW, Hixson JE: Linkage of serum insulin concentrations to chromosome 3p in Mexican Americans. *Diabetes* 49:513–516, 2000
5. An P, Teran-Garcia M, Rice T, Rankinen T, Weisnagel SJ, Bergman RN, Boston RC, Mandel S, Stefanovski D, Leon AS, Skinner JS, Rao DC, Bouchard C: Genome-wide linkage scans for prediabetes phenotypes in response to 20 weeks of endurance exercise training in non-diabetic whites and blacks: the HERITAGE Family Study. *Diabetologia* 48:1142–1149, 2005
6. Freedman BI, Rich SS, Sale MM, Heiss G, Djousse L, Pankow JS, Province MA, Rao DC, Lewis CE, Chen YD, Beck SR, HyperGEN Investigators: Genome-wide scans for heritability of fasting serum insulin and glucose concentrations in hypertensive families. *Diabetologia* 48:661–668, 2005
7. Chiu YF, Chuang LM, Hsiao CF, Hung YJ, Lin MW, Chen YT, Grove J, Jorgenson E, Quertermous T, Risch N, Hsiung CA: An autosomal genome-wide scan for loci linked to pre-diabetic phenotypes in nondiabetic Chinese subjects from the Stanford Asia-Pacific Program of Hypertension and Insulin Resistance Family Study. *Diabetes* 54:1200–1206, 2005
8. Mori Y, Otabe S, Dina C, Yasuda K, Populaire C, Lecoeur C, Vatin V, Durand E, Hara K, Okada T, Tobe K, Boutin P, Kadowaki T, Froguel P: Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate Loci on 7p and 11p. *Diabetes* 51:1247–1255, 2002
9. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ, Shepard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzch H, Schroder HE, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* 13:161–166, 1996
10. Pratley RE, Thompson DB, Prochazka M, Baier L, Mott D, Ravussin E, Sakul H, Ehm MG, Burns DK, Foroud T, Garvey WT, Hanson RL, Knowler WC, Bennett PH, Bogardus C: An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. *J Clin Invest* 101:1757–1764, 1998
11. Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow JJ, Birznieks G, Chang J, Eldridge W: The Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study. II. An autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–1200, 2000
12. An P, Hong Y, Weisnagel SJ, Rice T, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Chagnon YC, Bergman RN, Bouchard C, Rao DC: Genomic scan of glucose and insulin metabolism phenotypes: the HERITAGE Family Study. *Metabolism* 52:246–253, 2003
13. Rich SS, Bowden DW, Haffner SM, Norris JM, Saad MF, Mitchell BD, Rotter JI, Langefeld CD, Wagenknecht LE, Bergman RN: Identification of quantitative trait loci for glucose homeostasis: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 53:1866–1875, 2004
14. Tripathy D, Almgren P, Tuomi T, Groop L: Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care* 27:2204–2210, 2004
15. Hanson RL, Pratley RE, Bogardus C, Narayan KM, Roumain JM, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC: Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 151:190–198, 2000
16. Bergman RN, Zaccaro DJ, Watanabe RM, Haffner SM, Saad MF, Norris JM, Wagenknecht LE, Hokanson JE, Rotter JI, Rich SS: Minimal model-based insulin sensitivity has greater heritability and a different genetic basis than homeostasis model assessment or fasting insulin. *Diabetes* 52:2168–2174, 2003
17. Hong Y, Rice T, Gagnon J, Despres JP, Nadeau A, Perusse L, Bouchard C, Leon AS, Skinner JS, Wilmore JH, Rao DC: Familial clustering of insulin and abdominal visceral fat: the HERITAGE Family Study. *J Clin Endocrinol Metab* 83:4239–4245, 1998
18. Freedman BI, Rich SS, Sale MM, Heiss G, Djousse L, Pankow JS, Province MA, Rao DC, Lewis CE, Chen YD, Beck SR: Genome-wide scans for heritability of fasting serum insulin and glucose concentrations in hypertensive families. *Diabetologia* 48:661–668, 2005
19. Goodarzi MO, Taylor KD, Guo X, Quinones MJ, Cui J, Li X, Hang T, Yang H, Holmes E, Hsueh WA, Olefsky J, Rotter JI: Variation in the gene for muscle-specific AMP deaminase is associated with insulin clearance, a highly heritable trait. *Diabetes* 54:1222–1227, 2005
20. Wu KD, Hsiao CF, Ho LT, Sheu WH, Pei D, Chuang LM, Curb D, Chen YD, Tsai HJ, Dzau VJ, Cox D, Tai TY: Clustering and heritability of insulin resistance in Chinese and Japanese hypertensive families: a Stanford-Asian Pacific Program in Hypertension and Insulin Resistance sibling study. *Hypertens Res* 25:529–536, 2002
21. Henkin L, Bergman RN, Bowden DW, Ellsworth DL, Haffner SM, Langefeld CD, Mitchell BD, Norris JM, Rewers M, Saad MF, Stamm E, Wagenknecht LE, Rich SS: Genetic epidemiology of insulin resistance and visceral adiposity: the IRAS Family Study design and methods. *Ann Epidemiol* 13:211–217, 2003
22. Kandror KV, Pilch PF: Compartmentalization of protein traffic in insulin-sensitive cells. *Am J Physiol* 271:E1–E14, 1996
23. Easom RA: Beta-granule transport and exocytosis. *Semin Cell Dev Biol* 11:253–266, 2000
24. Huotari MA, Miettinen PJ, Palgi J, Koivisto T, Ustinov J, Harari D, Yarden Y, Otonkoski T: ErbB signaling regulates lineage determination of developing pancreatic islet cells in embryonic organ culture. *Endocrinology* 143:4437–4446, 2002
25. Ahlgren U, Pfaff SL, Jessell TM, Edlund T, Edlund H: Independent requirement for ISL1 in formation of pancreatic mesenchyme and islet cells. *Nature* 385:257–260, 1997
26. Hsueh WC, Mitchell BD, Aburomia R, Pollin T, Sakul H, Ehm MG, Michelsen BK, Wagner MJ, St. Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR: Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care* 23:595–601, 2000
27. Green P, Falls K, Crooks S: *Documentation for CRI-MAP. Version 2.4*. St. Louis, MO, Department of Genetics, School of Medicine, Washington University, 1990
28. Williams-Blangero S, Blangero J: Quantitative genetic analysis of skin reflectance: a multivariate approach. *Hum Biol* 64:35–49, 1992
29. Almasy L, Blangero J: Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62:1198–1211, 1998
30. Heath SC: Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. *Am J Hum Genet* 61:748–760, 1997