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

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Type 2 diabetes mellitus is a complex disorder encompassing multiple metabolic defects. We report results from an autosomal genome scan for type 2 diabetes–related quantitative traits in 580 Finnish families ascertained for an affected sibling pair and analyzed by the variance components-based quantitative-trait locus (QTL) linkage approach. We analyzed diabetic and nondiabetic subjects separately, because of the possible impact of disease on the traits of interest. In diabetic individuals, our strongest results were observed on chromosomes 3 (fasting C-peptide/glucose: maximum LOD score [MLS] = 3.13 at 53.0 cM) and 13 (body-mass index: MLS = 3.28 at 5.0 cM). In nondiabetic individuals, the strongest results were observed on chromosomes 10 (acute insulin response: MLS = 3.11 at 21.0 cM), 13 (2-h insulin: MLS = 2.86 at 65.5 cM), and 17 (fasting insulin/glucose ratio: MLS = 3.20 at 9.0 cM). In several cases, there was evidence for overlapping signals between diabetic and nondiabetic individuals; therefore we performed joint analyses. In these joint analyses, we observed strong signals for chromosomes 3 (body-mass index: MLS = 3.43 at 59.5 cM), 17 (empirical insulin-resistance index: MLS = 3.61 at 0.0 cM), and 19 (empirical insulin-resistance index: MLS = 2.80 at 74.5 cM). Integrating genome-scan results from the companion article by Ghosh et al., we identify several regions that may harbor susceptibility genes for type 2 diabetes in the Finnish population.

Introduction

Type 2 diabetes is a heterogeneous disease characterized by insulin resistance and β-cell dysfunction (De-

Fronzo 1987; Bergman 1989). Left untreated, the fasting hyperglycemia can lead to a variety of secondary complications, including retinopathy, nephropathy, and neuropathy. Type 2 diabetes is also a risk factor

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for cardiovascular disease and hypertension (DeFronzo and Ferrannini 1991; Harris 1990) and contributes significantly to disease morbidity and mortality. Although the clinical characteristics of type 2 diabetes are well documented, the specific physiologic or biochemical defects responsible for the pathogenesis of the disease remain unclear. There is good evidence for a genetic component to type 2 diabetes (Rich 1990; Ghosh and Schork 1996). It is also clear that environmental factors play a major role in disease pathogenesis.

Several groups have performed genome scans for type 2 diabetes, in attempts to identify disease-susceptibility loci (Hanis et al. 1996; Mahtani et al. 1996; Hanson et al. 1998; Imperatore et al. 1998; Pratley et al. 1998; Duggirala et al. 1999; Elbein et al. 1999; Ghosh et al. 1999; Ehm et al. 2000). Although evidence has been reported for chromosomal regions that may contain type 2 diabetes–susceptibility genes, until recently only genes for specific subtypes of type 2 diabetes, such as maturity-onset diabetes of the young (MODY), have appeared in the literature (Bell et al. 1991; Froguel et al. 1992; Vaxillaire et al. 1995; Horikawa et al. 1997; Stoffers et al. 1997). These relatively rare subtypes of type 2 diabetes have been determined to be monogenic in nature, and multiple studies suggest that these genes play, at most, a minor role in genetic susceptibility for the more general form(s) of type 2 diabetes (Hanis et al. 1996; Mahtani et al. 1996; Elbein et al. 1999). Recently, the chromosome 2 locus linked to type 2 diabetes in Mexican Americans (Hanis et al. 1996) has been cloned and identified as calpain-10 (Horikawa et al. 2000). This raises the possibility that additional type 2 diabetes–susceptibility genes might be cloned and identified in the near future.

In this article and the companion article (Ghosh et al. 2000 [in this issue]), we describe genomewide results from the Finland–United States Investigation of NIDDM Genetics (FUSION) study, a multicenter effort to positionally clone genes for type 2 diabetes in the Finnish population. As described in the companion article by Ghosh et al. (2000 [in this issue]), we recruited and tested 580 families ascertained for an affected sibling pair. An important component of FUSION was the measurement of a variety of type 2 diabetes–related quantitative traits in subjects with and without disease. As part of our efforts to identify disease-predisposing loci, we performed an autosomal genome scan using these disease-related traits. The rationale behind this approach is that, because the traits examined are closely related to type 2 diabetes, any quantitative-trait locus (QTL) that we identify may also act as a disease-susceptibility or modifier locus. Coupled with the results from our type 2 diabetes genome scan (Ghosh et al. 2000 [in this issue]), we identify along the genome

several regions that may harbor type 2 diabetes–susceptibility genes and that warrant further examination.

Subjects and Methods

Subjects and Phenotyping

The FUSION study design, recruitment, and phenotyping have been detailed elsewhere (Valle et al. 1998), and details regarding genotyping can be found in the companion article by Ghosh et al. (2000 [in this issue]). Therefore, in this article we only briefly review recruitment and phenotyping. Index cases were ascertained as those with age at onset of 35–60 years and at least one sibling diagnosed with type 2 diabetes. We successfully recruited and phenotyped 580 families, each with a minimum of one affected sibling pair (ASP) but, whenever possible, also collected additional siblings with type 2 diabetes. Two hundred ten of the families were also extended to include both a nondiabetic spouse and two or more nondiabetic offspring of the index case and/or diabetic siblings.

For all subjects, we collected basic demographic, anthropometric, and extensive medical-history information. Fasting glucose, insulin, lipids, and resting blood pressure were measured in all study subjects. Fasting C-peptide and glutamic acid decarboxylase antibody (GAD Ab) were measured in diabetic individuals, and these values were used in conjunction with insulin-treatment information to identify possible cases of late-onset type 1 diabetes, as described elsewhere (Valle et al. 1998). An oral glucose-tolerance test (OGTT), conforming to World Health Organization (1985) standards, was performed both in all nondiabetic subjects, to determine their glucose-tolerance status, and in diabetic subjects whose affection status could not be readily confirmed, yielding 2-h glucose and insulin measurements in these individuals. Because of the small number of diabetic individuals who underwent the OGTT, 2-h glucose and insulin data were analyzed only for the nondiabetic subjects.

Nondiabetic spouses and offspring of either index cases or diabetic siblings were also invited to undergo a tolbutamide-modified frequently sampled intravenous glucose-tolerance test (FSIGT), with Minimal Model analysis (Bergman et al. 1979; Steil et al. 1993), to derive quantitative estimates of glucose effectiveness (S_G) and insulin sensitivity (S_I). For these subjects, the acute insulin response to glucose (AIR) was computed as the incremental integrated area under the insulin curve for the first 8 min of the FSIGT and was used as an index of insulin secretion. We also computed the disposition index (DI), the product of S_I and AIR, as a resistance-

Table 1

Phenotypes

Sex	Diabetic Index Cases/Siblings ($n = 1,175$)	Nondiabetic Subjects	
		Spouses ($n = 194$)	Offspring ($n = 521$)
	Ratio (Female:Male)		
	599:576	132:62	261:260
	Mean ± SD [Median]		
Age (years)	64.2 ± 8.3 [64.8]	61.5 ± 7.7 [61.3]	34.6 ± 7.4 [34.8]
BMI (kg/m^2)	29.8 ± 4.8 [29.3]	28.4 ± 4.5 [28.1]	25.9 ± 4.5 [25.3]
WHR	.94 ± .08 [.94]	.88 ± .08 [.88]	.86 ± .08 [.85]
Fasting glucose (mM)	10.2 ± 3.5 [9.6]	5.2 ± .6 [5.2]	5.1 ± .6 [5.0]
2-h glucose (mM)	...	6.2 ± 1.8 [6.0]	5.3 ± 1.5 [5.2]
Fasting insulin (pM)	111 ± 67 [96]	76 ± 49 [66]	67 ± 35 [60]
2-h insulin (pM)	...	427 ± 315 [348]	317 ± 245 [228]
Fasting C-peptide (nM)	1.59 ± .93 [1.44]	...	...
$S_{I(EST)}$ (× 100)	.18 ± .31 [.11]	.35 ± .32 [.29]	.40 ± .34 [.34]
IR_I	12.0 ± 8.1 [10.3]	14.4 ± 8.2 [12.9]	13.2 ± 6.4 [12.3]
IR_C	.17 ± .11 [.14]	...	...
S_G (× 100 min^{-1})	...	1.65 ± .57 [1.59]	1.77 ± .58 [1.70]
S_I [× 10^{-5}min^{-1}/pM]	...	5.88 ± 3.41 [5.41]	7.57 ± 4.48 [6.71]
AIR (pM × 8 min)	...	2,331 ± 1,624 [1,868]	2,188 ± 1,528 [1,897]
DI	...	12,244 ± 8,726 [9,983]	14,178 ± 9,163 [12,538]

corrected index of β-cell function (Bergman et al. 1981; Kahn et al. 1993).

Empirical Metabolic Indices

Because we did not perform FSIGTs in our diabetic individuals, we could not scan for loci linked to the FSIGT-derived measures of insulin resistance and insulin secretion in these individuals. We attempted to circumvent this problem by constructing empirical indices for insulin resistance and insulin secretion, using the fasting-glucose, insulin, and C-peptide measurements from all our diabetic and nondiabetic subjects. For insulin resistance, we used the index described by Sluiter et al. (1976): $S_{I(EST)} = 1/(glucose × insulin)$. This empirical index for insulin resistance, $S_{I(EST)}$, was correlated with S_I from the Minimal Model, in nondiabetic spouses and offspring (Spearman correlation: $r = .54$; $P = .0001$).

Although fasting insulin or C-peptide might be considered reflections of insulin secretion, these measures do not account for the variability introduced by the underlying stimulatory effect of the fasting-glucose concentration. Thus, we constructed empirical indices for insulin secretion, as follows: IR_I = insulin/glucose and IR_C = C-peptide/glucose. Since our indices for insulin secretion are derived from fasting measurements, they likely reflect basal insulin secretion, rather than stimulated insulin secretion. Therefore, compared with the correlation between S_I and $S_{I(EST)}$, AIR was less strongly correlated with IR_I in the nondiabetic spouses and offspring (Spearman correlation: $r = .39$; $P = .0001$).

Fasting C-peptide was not measured in nondiabetic subjects; therefore, a comparison between AIR and IR_C was not possible. However, given the equimolar relationship between insulin and C-peptide (Rubenstein et al. 1969), we would expect the correlation to be similar.

Medication Exclusions

Prior to clinical testing, all subjects, diabetic and nondiabetic, were asked to refrain from taking any of their prescription medications during the day of clinic visits. Data for any subject who, on the morning of blood

Table 2

Analyses of Diabetic and Nondiabetic Subjects

	Diabetic Subjects	Nondiabetic Subjects
BMI	•	•
WHR	•	•
Fasting glucose	•	•
2-h glucose		•
Fasting insulin	•	•
2-h insulin		•
Fasting C-peptide	•	
$S_{I(EST)}$	•	•
IR_I	•	•
IR_C	•	
S_G		•
S_I		•
AIR		•
DI		•

Table 3

MLS ⩾1.18 in Diabetic Subjects

Position (cM)	Trait	Covariates[a]	MLS	P[b]
Chromosome 1:				
98.0	BMI	1	1.19	.0097
164.5	IR_C	1	1.45	.0049
Chromosome 3:				
21.0	IR_I	2	1.39	.0057
53.0	IR_C	2	3.13	.0001
56.0	BMI	1	1.19	.0097
58.0	C-peptide	2	2.26	.0006
Chromosome 4:				
28.5	IR_I	1	1.38	.0058
Chromosome 5:				
198.0	BMI	1	1.24	.0084
Chromosome 7:				
74.5	C-peptide	2	1.63	.0031
74.5	IR_C	2	1.76	.0022
101.0	Insulin	2	1.94	.0014
Chromosome 8:				
103.5	Glucose	1	1.23	.0042
Chromosome 9:				
0.0	Insulin	1	1.32	.0068
5.0	C-peptide	2	1.36	.0061
Chromosome 11:				
89.5	Insulin	2	2.07	.0010
91.0	IR_I	2	2.32	.0005
Chromosome 13:				
7.0	BMI	1	2.95	.0002
39.5	C-peptide	1	1.18	.0098
41.0	Insulin	1	1.22	.0090
Chromosome 14:				
96.5	$S_{I(EST)}$	2	1.19	.0096
102.0	Insulin	1	2.67	.0008
Chromosome 17:				
0.0	$S_{I(EST)}$	2	1.54	.0039
Chromosome 19:				
8.5	BMI	1	1.45	.0049
73.5	$S_{I(EST)}$	1	1.82	.0019

[a] 1 = age and sex; 2 = age, sex, and BMI.
[b] For MLS ⩾2.50, empirical P values from simulations are reported. For details, see the Subjects and Methods section.

sampling, took medications likely to affect the outcome of interest were excluded from analyses.

QTL Linkage Analysis

We used a multipoint variance-components approach for our QTL genome scan (Amos 1994; Blangero and Almasy 1997). We modeled the mean level of each quantitative-trait value as a linear function of age and sex. Because obesity is a known risk factor for type 2 diabetes (Lillioja and Bogardus 1988; Ohlson et al. 1988), and because genes for obesity may overlap with those for type 2 diabetes, we also tested models with additional adjustment for either body-mass index (BMI) or waist-to-hip ratio (WHR). Both BMI and WHR were also examined as quantitative traits. For this article, we chose

to report only those results from the age- and sex-adjusted and age-, sex-, and BMI-adjusted traits. We report results from either the unadjusted trait or the additional adjustment for WHR only when they provide notably stronger evidence for linkage.

The total variance (σ^2) for a quantitative trait was partitioned into components due to a major gene (σ^2_{mg}), additive polygenes (σ^2_{pg}), and individual-specific environment (σ^2_e): $\sigma^2 = \sigma^2_{mg} + \sigma^2_{pg} + \sigma^2_e$. Similarly, the covariance between the trait values for two noninbred individuals i and j was defined as $Cov(X_i, X_j) = \sigma^2_{mg} IBD_{ij} + \sigma^2_{pg} 2\phi_{ij} + \sigma^2_e \delta_{ij}$. Here, IBD_{ij} is the estimated proportion of genes shared identical by descent (IBD) by i and j at the putative major gene; ϕ_{ij} is the kinship coefficient, the probability that alleles drawn at random from individuals i and j, at a given locus, are IBD (Jacquard 1974); and $\delta_{ij} = 1$ if $i = j$ and $\delta_{ij} = 0$ if $i \neq j$.

All data were transformed to approximate univariate normality prior to analysis. Square root (in the case of C-peptide, DI, $S_{I(EST)}$, IR_I, and IR_C) or natural-logarithm transformations (in the case of fasting and 2-h glucose and insulin, S_I) were used for most variables. Exceptions were S_G ($y^{0.25}$) and AIR ($y^{0.185}$). Also, we used the natural log–transformed BMI and WHR values in the mean model, since their distributions were skewed. Maximum-likelihood parameter estimates were derived by use of the computer program USERFQTL. This software merges the IBD-estimation algorithm from S_IBLINK (Hauser and Boehnke 1998) with the variance-components algorithm from the computer program FISHER (Lange et al. 1988). The presence of a major trait gene was assessed at each evaluation point along the genome, by the likelihood-ratio statistic (Λ), in which we compared models with the major-gene variance component set to 0 against those in which it was allowed to be positive. Because of the one-sided nature of this test, Λ is asymptotically distributed as a 50:50 mixture of χ^2 on 1 df and a point mass at 0 (Self and Liang 1987), so that the P values are half as large as those in the two-sided case. For presentation purposes, in this article we have converted Λ to maximum LOD scores (MLSs).

Analyses were performed on nuclear families, with diabetic siblings analyzed separately from nondiabetic spouses and offspring, because of differences in trait distributions. In the case of nondiabetic-subject analyses, in which a diabetic index case or sibling served as a parent in the nuclear-family structure, the diabetic individual's genotype information was included for the purpose of IBD estimation, but his/her quantitative trait values were set to missing, so as not to contribute to the variance-covariance structure. In addition, we performed analyses on the offspring only, to protect against possible violations of model assumptions by inclusion of the nondiabetic spouses. In the majority of cases, the offspring-only analyses gave results similar to those of

Table 4

MLS ⩾1.18 in Nondiabetic Subjects

Position (cM)	Trait	Covariates	MLS	*P*
Chromosome 1:				
131.5	S_G	1	1.41	.0054
165.5	Glucose	1	1.67	.0028
265.0	Insulin	2	1.37	.0060
267.0	2-h insulin	2	2.63	.0003
Chromosome 2:				
97.0	S_G	1	1.22	.0090
145.0	S_I	2	1.43	.0051
Chromosome 3:				
38.0	DI	1	1.31	.0071
40.0	Glucose	2	2.22	.0008
65.0	BMI	1	2.16	.0008
Chromosome 6:				
41.0	Glucose	2	1.92	.0015
69.0	BMI	1	1.18	.0100
119.5	Insulin	1	1.85	.0019
119.5	IR_I	1	2.02	.0012
Chromosome 7:				
0.0	Glucose	2	1.24	.0085
82.0	BMI	1	2.21	.0007
Chromosome 8:				
13.0	DI	1	1.32	.0069
135.5	BMI	1	2.29	.0006
Chromosome 9:				
51.0	2-h insulin	2	1.19	.0097
114.5	AIR	2	1.97	.0013
Chromosome 10:				
12.5	Glucose	1	1.23	.0087
21.0	AIR	2	3.11	.0001
102.0	S_G	1	1.63	.0031
Chromosome 12:				
129.5	BMI	1	1.36	.0061
Chromosome 13:				
62.0	IR_I	2	1.37	.0059
65.5	2-h insulin	1	2.86	.0002
Chromosome 14:				
0.0	DI	1	1.19	.0096
1.0	S_I	2	1.46	.0047
5.5	2-h insulin	2	1.51	.0042
25.5	Glucose	1	2.01	.0012
Chromosome 15:				
74.0	$S_{I(EST)}$	1	1.18	.0100
74.0	Insulin	2	1.53	.0040
75.0	IR_I	2	1.48	.0045
Chromosome 16:				
42.0	$S_{I(EST)}$	1	1.47	.0046
44.5	IR_I	2	1.89	.0016
45.0	Insulin	2	1.71	.0025
65.0	2-h insulin	1	1.28	.0077
78.0	S_G	1	1.33	.0067
Chromosome 17:				
0.0	2-h glucose	2	1.44	.0051
9.0	Insulin	1	2.97	.0001
9.0	2-h insulin	2	2.71	.0006
9.0	$S_{I(EST)}$	1	2.25	.0006
9.0	IR_I	1	3.20	<.0001
46.5	S_G	1	1.27	.0077
Chromosome 18:				
36.0	WHR	1	2.61	.0003
Chromosome 19:				
72.5	2-h insulin	1	1.39	.0057
74.0	IR_I	1	1.52	.0040
75.5	Insulin	1	1.38	.0058
76.5	$S_{I(EST)}$	1	1.94	.0014
79.5	2-h glucose	1	1.40	.0065
94.5	Glucose	1	2.05	.0011
Chromosome 20:				
9.5	2-h glucose	2	1.76	.0022
19.5	IR_I	1	1.19	.0095
Chromosome 22:				
0.0	2-h insulin	1	2.08	.0010

NOTE.—Data are as defined in the footnotes to table 3.

the nondiabetic spouses and offspring together. Thus, we report only results from the combined nondiabetic spouse and offspring analyses.

We made the a priori decision to analyze diabetic and nondiabetic subjects separately, because of differences in the trait distributions in the two groups. These differences are likely due either to the traits being influenced by the diabetes per se or to the variety and variable efficacy of treatment regimens that the diabetic subjects were undergoing at the time of testing. However, for several traits, we observed overlapping linkage signals between diabetic and nondiabetic subjects. For these situations, we combined the diabetic and nondiabetic trait data and examined whether the trait distributions were approximately univariate normal (data not shown). If the combined trait distribution was approximately univariate normal, we combined the data from diabetics and nondiabetics in a single joint analysis. In these joint analyses, we included disease affection status as an additional covariate in our models. In addition, because USERFQTL limits the analysis to nuclear families, the extended family structure was broken down into individual nuclear families for analysis.

Statistical Significance

Lander and Kruglyak (1995) have proposed guidelines for interpretation of linkage results from genomewide scans. As such, their recommendations are applicable only for interpretation on a genomewide level. Furthermore, their simulations were designed to assess linkage based on affection status and not for QTLs. Therefore, we performed computer simulations to assess pointwise statistical significance more accurately for our most interesting results (MLS ⩾2.50). In each replicate sample, marker genotypes were simulated under the null hypothesis of no linkage, with the observed nuclear-family structures and phenotypes. Simulations of 10,000 or 100,000 replicates were generated, and the resulting LOD-score distribution was used to generate empirical *P* values. In general, the empirical *P* values were slightly larger than the nominal *P* values, suggesting that the nominal values tend to slightly overstate the evidence

for linkage (data not shown). Thus, for MLS $\geqslant 2.50$ we report the empirical P values from our simulations.

Results

The mean, median, and SD of trait values for subjects analyzed for this QTL genome scan are shown in table 1. Table 2 provides a breakdown of analyses, by quantitative trait and group. As noted above, neither FSIGT-derived traits for diabetic subjects nor fasting C-peptide and, consequently, IR_C, for nondiabetic subjects was available for analysis.

Tables 3 and 4 summarize MLS $\geqslant 1.18$ (corresponding to $P < .01$) for diabetic and nondiabetic subjects, respectively. If more than one model yielded a result greater than this cutoff for a given trait, we report only the result with the highest MLS. For 13% of the results reported in tables 3 and 4, one of the alternative models using different covariates yielded an MLS that was both either lower or higher than the value reported in the table and above the 1.18 MLS cutoff value.

Diabetic Subjects

In the analyses of our diabetic subjects, the strongest evidence for QTL linkage was observed on chromosomes 3 and 13, for IR_C and BMI, respectively (fig. 1). On chromosome 3 (fig. 1A), the MLS for age-, sex-, and BMI-adjusted IR_C was 3.13 at 53.0 cM, between markers D3S1266 and D3S1768, and had a 1-LOD support interval of 44.0–67.0 cM. As might be expected, because it is a component of IR_C, age-, sex-, and BMI-adjusted fasting C-peptide also showed evidence for linkage (MLS = 2.26) in the same region of chromosome 3, and it yielded a maximum at 58.0 cM near marker D3S1768.

On chromosome 13 (fig. 1B), the MLS for BMI was 3.28 at 5.0 cM, between markers D13S175 and D13S221, and had a 1-LOD support interval of 0.0–16.0 cM. With adjustment for age and sex, this signal dropped to an MLS of 2.95 at 7.0 cM. There were no other linkage peaks in this region of the chromosome, for any of the other traits. A second region along chromosome 13 (fig. 1B) showed more-modest evidence for linkage to fasting insulin (MLS = 1.52 at 41.0 cM), $S_{I(EST)}$ (MLS = 1.47 at 41.5 cM), and age-, sex-, and WHR-adjusted fasting C-peptide (MLS = 1.23 at 40 cM). All three signals maximized near marker D13S263.

Other noteworthy results include those for age- and sex-adjusted fasting insulin on chromosome 14 (MLS = 2.67 at 102.0 cM, near marker D14S95), as well as both IR_I (MLS = 2.32 at 91.0 cM, near marker D11S1311) and age-, sex-, and BMI-adjusted fasting insulin, on chro-

mosome 11. The MLS curve for fasting insulin maximized at 90.0 cM (MLS = 2.26 near D11S1311), and there was a secondary peak at 53.5 cM (MLS = 1.52). When $S_{I(EST)}$ was examined, this secondary peak showed evidence for linkage (MLS = 1.68). Given that $S_{I(EST)}$ is derived on the basis of fasting insulin, this suggests that on this chromosome there may be a QTL involved in some complex interaction between insulin and glucose.

Nondiabetic Subjects

In the analysis of the nondiabetic spouses and offspring, the strongest evidence for linkage was observed on chromosomes 10, 13, and 17 (fig. 2). On chromosome 10 (fig. 2A), the MLS for age-, sex-, and BMI-adjusted AIR was 3.11 at 21.0 cM, near marker D10S1720, with a 1-LOD support interval of 13.0–27.5 cM. Fasting glucose also showed a weaker linkage peak in the same region (MLS = 1.38 at 21.0 cM). Unadjusted fasting insulin and the empirical indices IR_I, IR_C, and $S_{I(EST)}$ all showed very modest linkage peaks (MLS = 0.58–0.89) in the same region (data not shown).

On chromosome 13, the MLS for age- and sex-adjusted 2-h insulin was 2.86 at marker D13S156 at 65.5 cM (fig. 2B); however, the 1-LOD support interval was very wide (50.0–76.5 cM). Age-, sex-, and BMI-adjusted IR_I also showed evidence for linkage in the same region (MLS = 1.37 at 62.0 cM). On chromosome 17 (fig. 2C), age- and sex-adjusted IR_I showed the strongest evidence for linkage, with MLS = 3.20 at 9.0 cM (1-LOD support interval 4.0–15.0 cM), near marker D17S1832. Not surprisingly, age- and sex-adjusted fasting insulin (MLS = 2.97) and $S_{I(EST)}$ (MLS = 2.25) both showed evidence for linkage in this region. However, age-, sex-, and BMI-adjusted 2-h insulin also showed evidence for linkage at the same location (MLS = 2.71), with a 1-LOD support interval of 0.5–13.5 cM (fig. 2C). Other noteworthy results in our nondiabetic subjects include 2-h insulin, on chromosome 1 (MLS = 2.70 at 270.0 cM); BMI, on chromosome 3 (MLS = 2.52 at 64.0 cM); age- and sex-adjusted WHR, on chromosome 18 (MLS = 2.61 at 36.0 cM); and age-, sex-, and WHR-adjusted 2-h insulin, on chromosome 22 (MLS = 2.70 at 0.0 cM).

Overlap of Results for Diabetic and Nondiabetic Subjects

We observed some regions where evidence for linkage to a specific quantitative trait overlapped between diabetic and nondiabetic subjects. Specifically, on chromosome 3 we observed a stronger signal (MLS = 2.52 at 64.0 cM) for BMI in our nondiabetic subjects and a weaker signal in the same proximity in our diabetic subjects (MLS = 1.19 at 56.0 cM), after adjustment for
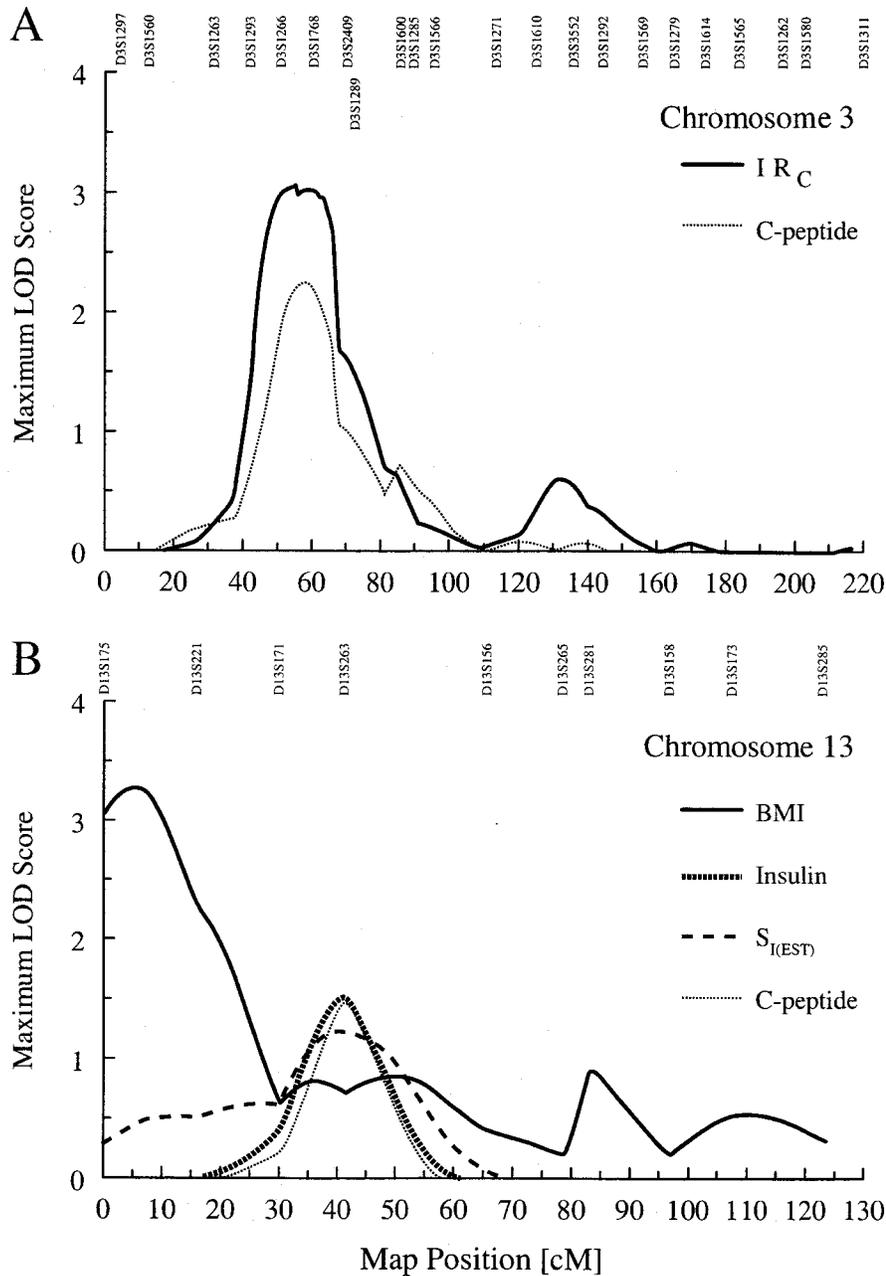
Figure 1   *A,* MLS curves for age-, sex-, and BMI-adjusted IR_C and for age-, sex-, and BMI-adjusted fasting C-peptide, for chromosome 3 in diabetic subjects. *B,* MLS curves for results for chromosome 13 in diabetic subjects. Unadjusted BMI showed the strongest evidence for linkage. However, a cluster of other traits—fasting insulin; $S_{I(EST)}$; and age-, sex-, and WHR-adjusted fasting C-peptide—showed weaker evidence for linkage at a different locus. Marker names and positions are given at the top of each graph.

age and sex. We also observed a similar situation with $S_{I(EST)}$ on chromosomes 17 and 19. For these chromosomes, we repeated QTL linkage analyses for these traits, combining data from both groups in a joint analysis.

In the joint analysis of all subjects for BMI without adjustment for covariates on chromosome 3, we observed an MLS of 3.43 at 59.5 cM (fig. 3*A*); when we

adjusted for age and sex, the MLS dropped to 2.83. Similarly, for $S_{I(EST)}$ on chromosome 17, when we combined our diabetic and nondiabetic subjects in a joint analysis, we observed an MLS of 3.61 at 0.0 cM, after adjusting for age, sex, and BMI (fig. 3*B*). The strong telomeric signal suggests the need for additional markers in this region, to refine our linkage signal. For chro-
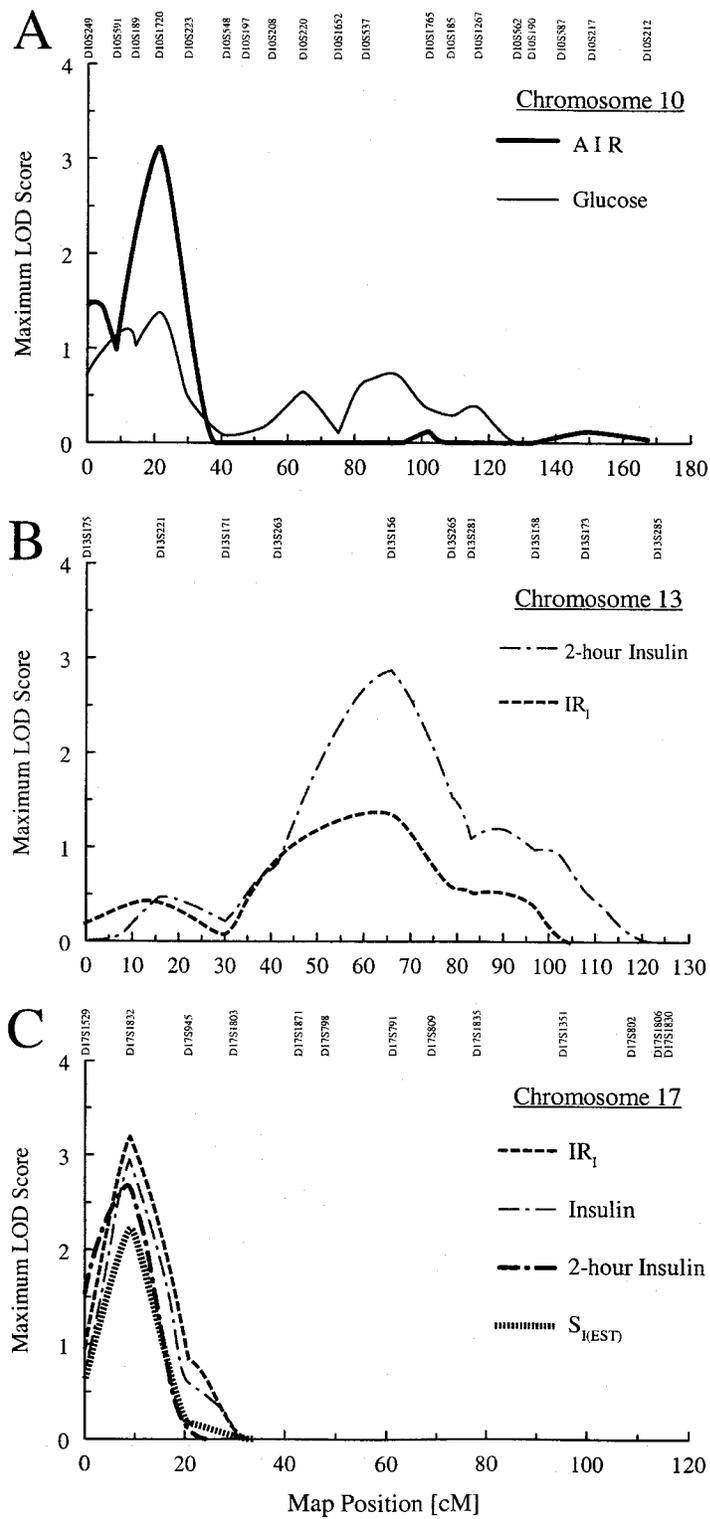
Figure 2 *A*, MLS curves for age-, sex-, and BMI-adjusted AIR and for fasting glucose, for chromosome 10 in nondiabetic subjects. *B*, MLS curves for age- and sex-adjusted 2-h insulin and for age-, sex-, and BMI-adjusted IR_I, for chromosome 13 in nondiabetic subjects. *C*, MLS curves for age- and sex-adjusted IR_I, age- and sex-adjusted fasting insulin, for age-, sex-, and BMI-adjusted 2-h insulin, and for age- and sex-adjusted $S_{I(EST)}$, for chromosome 17 in nondiabetic subjects.
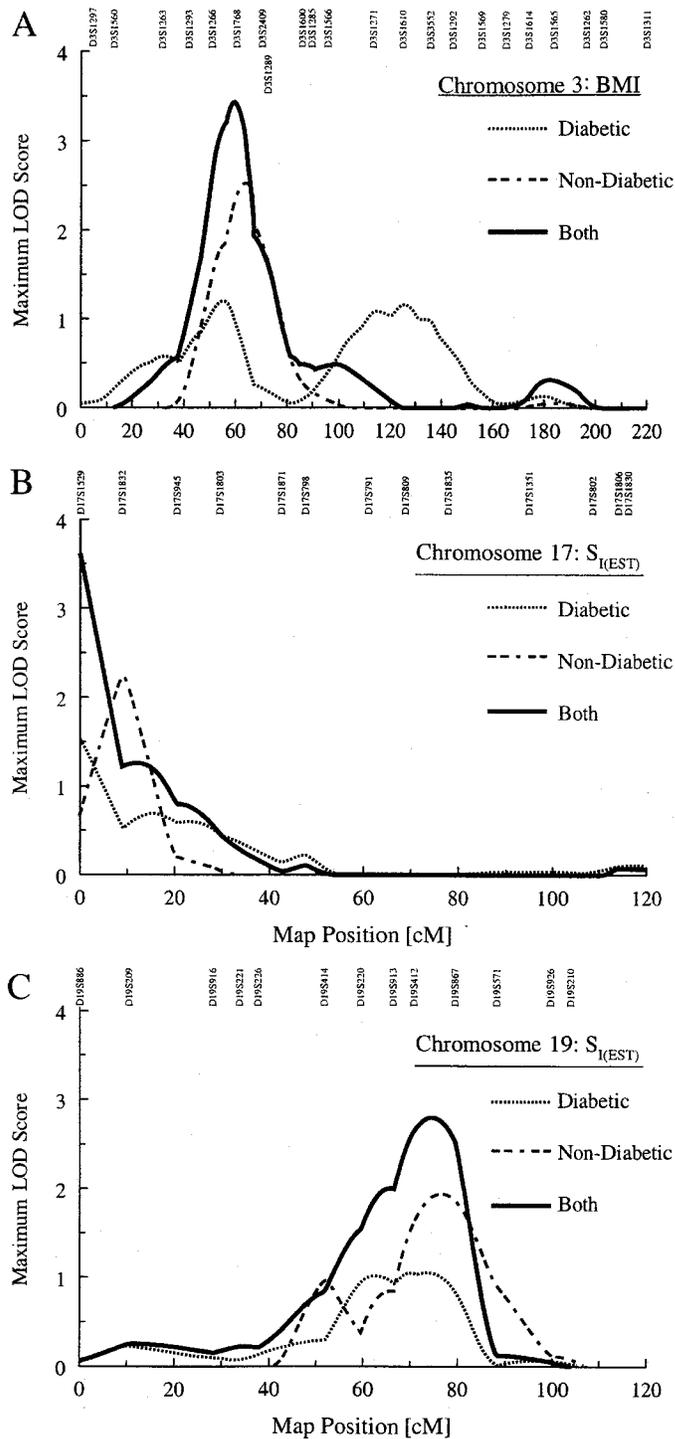
Figure 3 *A,* MLS curves for age- and sex-adjusted BMI in diabetic subjects and for BMI in nondiabetic subjects, for chromosome 3. When data from both groups are analyzed jointly, BMI shows a strong signal at 59.5 cM, with an MLS of 3.43. *B,* MLS curves for age-, sex-, and BMI-adjusted $S_{I(EST)}$ in diabetic subjects and for age- and sex-adjusted $S_{I(EST)}$ in nondiabetic subjects, for chromosome 17. Joint analysis of age-, sex-, and BMI-adjusted $S_{I(EST)}$ shows a stronger signal (MLS = 3.61) at the p-terminus. *C,* MLS curves for age- and sex-adjusted $S_{I(EST)}$, in both diabetic and nondiabetic subjects, for chromosome 19. Joint analysis of age-, sex-, and WHR-adjusted $S_{I(EST)}$ shows a stronger signal (MLS = 2.80) at 74.5 cM.

mosome 19, the joint analysis yielded an MLS of 2.80 at 74.5 cM after adjustment for age, sex, and WHR (fig. 3C).

There were other examples of overlapping signals across traits. On chromosome 1, the signal for age- and sex-adjusted IR_C in the diabetic subjects (MLS = 1.61) and fasting glucose for the same adjustment in the nondiabetics (MLS = 1.66) both maximized in the same region (~165 cM). Similarly, on chromosome 3, age-, sex-, and BMI-adjusted fasting C-peptide in the diabetics (MLS = 2.26) and age- and sex-adjusted BMI in the nondiabetics (MLS = 2.52) maximized in close proximity, at 58.0 and 64.0 cM, respectively. Chromosome 19 showed a particularly interesting set of four outcomes: the evidence for linkage to age- and sex-adjusted $S_{I(EST)}$ in diabetic subjects (MLS = 1.82 at 73.5 cM) overlapped with those for 2-h insulin (MLS = 1.59 at 71.0 cM) in the nondiabetic subjects, age- and sex-adjusted $S_{I(EST)}$ (MLS = 1.94 at 76.5 cM) in the nondiabetic subjects, and age- and sex-adjusted IR_I (MLS = 1.52 at 74.0 cM) in the nondiabetic subjects.

Discussion

Type 2 diabetes has all the characteristics of a complex disorder: convincing evidence for a genetic component, ambiguous mode of inheritance, strong environmental component, and likely multiple genes of varying effect that work independently and/or in concert (Rich 1990; Ghosh and Schork 1996). The difficult task of identifying the susceptibility genes is complicated by the fact that the interaction of these factors results in a variety of disease etiologies. Thus, although, because of clearly defined diagnostic criteria (National Diabetes Data Group 1979; World Health Organization 1985), individuals can be identified as having type 2 diabetes, not all individuals who eventually develop type 2 diabetes follow the same path toward disease, nor will they follow the same progression toward disease complications. These complex features may account for why, despite the large number of groups performing genomic scans for type 2 diabetes (Hanis et al. 1996; Mahtani et al. 1996; Hanson et al. 1998; Imperatore et al. 1998; Pratley et al. 1998; Duggirala et al. 1999; Elbein et al. 1999; Ghosh et al. 1999; Ehm et al. 2000), there have been, until recently, no published reports of a successful cloning and identification of a major susceptibility gene. However, recently the chromosome 2 locus linked to type 2 diabetes in Mexican Americans, also known as "NIDDM1" (Hanis et al. 1996), has been cloned and identified as calpain-10, a calpain-like cysteine protease (Horikawa et al. 2000).

In this article and the companion article (Ghosh et al. 2000 [in this issue]), we have reported the initial results of a genomewide scan for linkage to diabetes and diabetes-related quantitative traits in the FUSION study. In the companion article (Ghosh et al. 2000 [in this issue]), we have reported results from our genomewide scan of affected sibling pairs sampled from the population of Finland. Given the expected difficulties in doing a genome scan for a complex disease, we augmented our ASP linkage results by doing disease-by-marker association analysis, ordered-subsets linkage analysis of the disease status data, and QTL linkage analysis of our quantitative-trait data. In this discussion, we focus on the results from our QTL genome scan but also draw on results from our other analyses, where our evidence for a possible diabetes-susceptibility gene may be the strongest.

The goal of QTL linkage analysis is to identify loci that influence specific quantitative traits; in our study, the traits that we examined are also intimately associated with type 2 diabetes. Through this approach, we hoped to identify QTLs that might also act as diabetes-susceptibility loci. We identified along the genome several regions that may contain QTLs. In our analytical strategy, we chose to analyze data from diabetic and nondiabetic subjects separately. There were two reasons for taking this approach. First, the characteristics of the trait distribution between diabetic and nondiabetic subjects tended to be different. This likely reflects the possibility that the trait may behave differently in diabetic individuals, because of disease-induced alterations in physiology and/or biochemistry. In addition, >90% of our diabetic volunteers were being treated for their disease through pharmacological intervention, and a large proportion of the remainder were treated via lifestyle modification—that is, changes in diet and/or physical activity. Thus, trait information in these subjects is likely to be confounded by these treatment effects.

Despite these potential differences, we did observe overlapping signals for certain traits across the two groups. We further investigated these traits by combining data from diabetic and nondiabetic subjects after examining the trait distribution of the joint data. In the joint analyses, we observed substantial increases in some of our overall MLSs (fig. 3).

A secondary consideration is the issue of overlapping linkage signals across different traits. We found these overlaps of particular interest, since many of the traits that we examined in FUSION, although clinically or physiologically important, still reflect the net integrated effect of multiple downstream biochemical events. Furthermore, because many of these traits are part of the endocrine hormone–feedback regulatory system, it would not be unusual to see a single locus having pleiotropic effects on two or more traits. Thus, we believe that overlapping signals across traits should be carefully

evaluated, taking into account whether such clustering of traits tells a coherent story.

A large proportion of our linkage results involved traits associated with β-cell function: fasting insulin, fasting C-peptide, 2-h insulin, and AIR. This suggests that genes associated with β-cell function may be exerting the strongest genetic effects in the population that we studied, and it is consistent with our observation that, in our sample, heritability for secretion-related traits was stronger than that for insulin resistance–related traits (Watanabe et al. 1999). This pattern was also found in a twin study in the same population (Lehtovirta et al. 2000).

Both the National Institutes of Health (NIH) group in Phoenix that is studying the Pima Indians (Pratley et al. 1998) and the group studying the Old Order Amish (St. Jean et al. 1999) have completed QTL genome scans for type 2 diabetes–related quantitative traits, and the Phoenix group has completed linkage analyses for diabetic nephropathy and retinopathy (Imperatore et al. 1998). Also, several groups have reported QTL linkage results for specific chromosomes (Thompson et al. 1995; Duggirala et al. 1996, 1999; Stern et al. 1996; Comuzzie et al. 1997). In what follows, we note regions along the genome where evidence for linkage in our QTL genome scan appears to overlap with evidence reported by these different studies and where results from our own ASP linkage analyses provide additional evidence for regions potentially harboring type 2 diabetes–susceptibility genes.

On chromosome 3 in Pima Indians, two peaks for fasting insulin (MLS = 1.30 at 35 cM and MLS = 1.25 at 45 cM) that flank our linkage peak (MLS = 2.22) for fasting glucose at 40 cM in nondiabetic subjects have been reported (Pratley et al. 1998). Also, the GENNID study recently reported evidence for linkage (MLS = 3.91) to type 2 diabetes in Mexican Americans, at marker D3S2432 (∼50 cM) (Ehm et al. 2000). We also have observed weaker linkage signals for BMI (MLS = 1.27 at 55.0 cM) in our diabetic subjects and for both AIR (MLS = 1.12 at 47.0 cM) and DI (MLS = 1.31 at 38.0 cM) in our nondiabetic subjects. This clustering of insulin secretion–related traits in our sample suggests that this QTL may be involved in the regulation of β-cell function.

The gene for peroxisome-proliferator-activator receptor-γ2 (PPARG [MIM 601487]) is near this region of chromosome 3. PPARG plays an important role in adipogenesis and insulin sensitization (Auwerx 1999). Recent reports, including our own study (J. A. Douglas, M. R. Erdos, R. M. Watanabe, A. Braun, C. L. Johnston, P. Oeth, K. L. Mohlke, T. T. Valle, C. Ehnholm, T. A. Buchanan, R. N. Bergman, F. S. Collins, M. Boehnke, and J. Tuomilehto, unpublished data), have shown an association between the presence of the Pro12Ala polymor-

phism in PPARG and both type 2 diabetes (Deeb et al. 1998) and obesity (Beamer et al. 1998).

We also observed linkage signals proximal to the PPARG region. These include fasting C-peptide (MLS = 2.26 at 58.0 cM) and IR$_\text{C}$ (MLS = 3.13 at 53.0 cM) in our diabetic subjects and BMI (MLS = 2.52 at 64.0 cM) in our nondiabetic subjects. In Pima Indians, peaks for fasting insulin (MLS = 1.22 at 188 cM [Pratley et al. 1998]) and for diabetic nephropathy (MLS = 2.03 at 181.1 cM [Imperatore et al. 1998]) have been reported near the signal that we found for S$_\text{I(EST)}$ (MLS = 1.59) at 196 cM. This region includes the gene for somatostatin (SST [MIM 182450]).

The Phoenix group also has reported, on chromosome 6, possible linkage to fasting glucose (MLS = 1.79 at 41 cM [Pratley et al. 1998]), which coincides with our evidence for linkage (MLS = 1.92) for the same trait and chromosomal location (table 4) in our nondiabetic subjects. Stern et al. (1996) have reported linkage to fasting glucose, on chromosome 6 at D6S300, ∼50 cM away from the location of our linkage peak, toward the q-terminus. This 40–50-cM region of chromosome 6 includes a variety of plausible candidate genes, including those for the major histocompatibility complex, tumor necrosis factor-α (TNF [MIM 191160]), and glucagon-like peptide-1 receptor (GLP1R [MIM 138032]).

In our nondiabetic subjects, we also had, for both fasting insulin (MLS = 1.85 at 119.5 cM) and IR$_\text{I}$ (MLS = 2.02 at 119.5 cM), interesting QTL results that overlapped with those of our ASP-based linkage analysis (MLS = 0.61-0.78 near 113 cM) (Ghosh et al. 2000 [in this issue]). Ordered-subsets analysis revealed two subsets with strong evidence for linkage to this region: low age at diagnosis (74 families; MLS = 2.48 at 104.5 cM) and low fasting glucose (94 families; MLS = 3.17 at 117.0 cM). In addition, in our nondiabetic subjects, we observed weaker QTL linkage results for 2-h insulin (MLS = 1.16 at 128.0 cM) and S$_\text{I(EST)}$ (MLS = 1.17 at 125.0 cM). Stern et al. (1996) have reported, near this region of chromosome 6 in their sample of Mexican Americans, evidence for linkage to fasting glucose (MLS = 2.17). Also, linkage to age-adjusted type 2 diabetes has been reported in this region (Z-score = 1.39 at 128 cM) in the Pima Indians (Hanson et al. 1998).

The Phoenix group has reported evidence for linkage, on chromosome 9, to both 2-h insulin (MLS = 2.16 at 98 cM [Pratley et al. 1998]) and diabetic nephropathy (two-point MLS = 1.28 at 114.6 cM [Imperatore et al. 1998]), near our peak (MLS = 1.97 at 114.5 cM) for AIR. This region includes the genes for muscle and hepatic fructose-1,6 bisphosphatase (FBP2 [MIM 603027] and FBP1 [MIM 229700]), a key rate-limiting enzyme for gluconeogenesis.

In the region on chromosome 19 where we observed

evidence for linkage to multiple traits, Pratley et al. (1998) have reported linkage to 2-h glucose (MLS = 1.14 at 75 cM). We also observed a weak association between marker D19S867 at 79.5 cM and affection status (P = .05; Ghosh et al. 2000 [in this issue]). This region includes genes for the muscle form of creatine kinase (CKM [MIM 123310]), gastric inhibitory peptide receptor (GIPR [MIM 137241]), hepatocyte nuclear factor 3γ (HNF3G [MIM 602295]), and muscle glycogen synthase I (GYS1 [MIM 138570]). GYS1 is the rate-limiting step for glycogen formation in muscle and has been examined as a candidate gene, by both Group et al. (1993) and the Phoenix group (Majer et al. 1996). Group et al. (1993) noted a specific allele that identified a subgroup of their diabetic subjects with both a strong family history of disease and a higher prevalence of hypertension and insulin resistance. In contrast, Majer et al. (1996) found no mutations in the GYS1 gene or alterations in mRNA expression or enzymatic activity.

Elsewhere, we have reported, for chromosome 20, modest QTL results (Ghosh et al. 1999) that overlapped with the ASP linkage peak toward the p-terminus of the chromosome. We have since genotyped an additional 5 markers, bringing the total to 43 markers genotyped on chromosome 20. The information provided by these markers modestly increased our QTL linkage signals for 2-h insulin (from MLS = 1.06 at 19.0 cM to MLS = 1.09 at 18.5 cM), IR_I (from MLS = 1.12 at 19.5 cM to MLS = 1.19 at 19.5 cM), and 2-h glucose (from MLS = 1.59 at 9.5 cM to MLS = 1.70 at 9.0 cM).

Finally, on the p-terminus of chromosome 22, the Phoenix group reports evidence for linkage to high-dose M-value from the glucose clamp (MLS = 2.12), corresponding to our result for 2-h insulin (MLS = 2.70). We are not aware of any obvious candidate genes in this region.

We identified other candidate genes that, on the basis of our QTL genome scan, fall into regions of interest. Many, if not all, have been examined as candidate genes in a variety of type 2 diabetes populations. On chromosome 1, the gene for pyruvate kinase (PK1 [MIM 266200]), a key glycolytic enzyme, and the gene for the G-protein coupled potassium channel (KCNJ9 [MIM 600932], also known as "GIRK3" or "Kir3.3") fall within the region in which we observed evidence for linkage both to IR_C in our diabetic subjects (MLS = 1.61) and to fasting glucose in our nondiabetic subjects (MLS = 1.66). Genes for insulin-like growth factor–binding proteins 1 and 3 (IGFBP1 [MIM 146730] and IGFBP3 [MIM 146732]) are near the chromosome 7 region in which we observed results, in diabetic subjects, for fasting C-peptide (MLS = 1.63) and IR_C (MLS = 1.76). IGF-binding proteins regulate IGF activity and, under some conditions, may play a role in devel-

opment of diabetic complications (Bach and Rechler 1995). Glucokinase (GCK [MIM 138079]), the key rate-limiting enzyme for glycolysis, is found just proximal to this region, and polymorphisms in this gene are responsible for MODY-2 (Froguel et al. 1992; Vionnet et al. 1992) St. Jean et al. (1999) have noted, on chromosome 14, evidence for linkage to fasting glucose (MLS = 2.00 at 29 cM) and to 2-h glucose (MLS = 2.53 at 22 cM), in large pedigrees from the Old Order Amish. These results coincide with our fasting-glucose result (MLS = 2.28 at 24.5 cM) in nondiabetic subjects. The genes for phosphoenolpyruvate carboxykinase 2 (PCK2 [MIM 261650]) and hepatocyte nuclear factor 3α (HNF3A [MIM 602294]) flank this region of chromosome 14. Finally, on chromosome 17, genes for tyrosine kinase nonreceptor 1, glucose transporter 4 (SLC2A4 [MIM 138190]), mitogen-activated protein kinase kinase 4 (MAP2K4 [MIM 601335]), and adenosine A2b receptor (ADORA2B [MIM 600446]) fall into the region of our linkage peaks for fasting insulin (MLS = 2.97), 2-h insulin (MLS = 2.71), $S_{I(EST)}$ (MLS = 2.25), and IR_I (MLS = 3.20), at 9.0 cM in our nondiabetic subjects.

In summary, we report the results from both a type 2 diabetes genome scan (Ghosh et al. 2000 [in this issue]) and an autosomal genome scan for type 2 diabetes–related quantitative traits. Our QTL linkage analyses reveal along the genome several regions that may include genes related to both adiposity and insulin secretion in our Finnish cohort. Furthermore, there is overlap between our QTL linkage results and results from our disease status–based linkage results. When we integrated the results from all of our analyses, we identified, on chromosomes 1–3, 6, 7, 10, 11, 17, 19, and 20, regions that have evidence for possible diabetes-susceptibility loci. We have now targeted these regions for further statistical analyses and/or additional genotyping, to further refine our evidence for linkage.

Acknowledgments

Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www3 .ncbi.nlm.nih.gov/Omim (for PPARG [MIM 601487], SST [MIM 182450], TNF [MIM 191160], GLP1R [MIM 138032], FBP1 [MIM 229700], FBP2 [MIM 603027], CKM [MIM 123310], GIPR [MIM 137241], HNF3G [MIM 602295], GYS1 [MIM 138570], PK1 [MIM 266200], KCNJ9 [MIM 600932], IGFBP1 [MIM 146730], IGFBP3 [MIM 146732], GCK [MIM 138079], PCK2 [MIM 261650], HNF3A [MIM 602294], SLC2A4 [MIM 138190], MAP2K4 [MIM 601335], and ADORA2B [MIM 600446])

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