Linkage Studies of NIDDM With 23 Chromosome 11 Markers in a Sample of Whites of Northern European Descent

Steven C. Elbein, Kelli L. Bragg, Michael D. Hoffman, Rachel A. Mayorga, and Mark F. Leppert

Considerable data support a genetic basis to susceptibility for NIDDM, but previous analysis of candidate genes has failed to identify a major susceptibility locus. Among regions with multiple potential candidates is chromosome 11, which includes the apolipoprotein C3 cluster, muscle glycogen phosphorylase, two insulin-dependent diabetes loci, the sulfonylurea receptor, and ataxia telangiectasia. To test linkage, we initially typed 19 markers at 10- to 15-cM intervals along chromosome 11. Analyses carried out under parametric models in members of 16–19 families of northern European ancestry detected possible linkage of NIDDM to D11S916. Nonparametric methods detected possible linkage to NIDDM at D11S901, which was 5–10 cM distant, and at D11S935, which was ~30 cM distant. Both D11S916 and D11S901 were near the IDDM4 locus. To further test linkage, we typed five additional markers within 5 cM of D11S916 in the initial 19 families. We also tested markers from the linked region in a second set of recently sampled additional families. Two additional markers (D11S527 and D11S534) showed possible linkage in the initial 19 families, but none of the markers were linked to NIDDM in a separate set of families from the same ethnic background. The best evidence for linkage in the combined data set of the initial 19 families and 26 additional families was at D11S534 under parametric analysis (Z = 1.20) and at D11S935 under nonparametric analysis (affected pedigree member, P = 0.0013). Our findings suggest marginal evidence for a diabetes susceptibility locus in the region between D11S901 and D11S935, with the best evidence for a locus at or near D11S935. Replication of these findings in other populations will be necessary to distinguish false-positive linkage from a true NIDDM susceptibility locus. *Diabetes* 45:370–375, 1996

A high concordance in identical twins, familial clustering of diabetic individuals, and predisposing pathophysiological abnormalities in offspring and relatives of diabetic individuals provide convincing evidence for an inherited susceptibility to NIDDM (1). Based on these data, an extensive search has been undertaken to identify defects in genes that code for proteins in the pathways of insulin secretion and peripheral insulin-stimulated glucose uptake. Despite the identification of rare mutations, no known mutation is likely to explain the inherited predisposition to NIDDM (1). The development of a dense map of highly informative markers (2,3) makes an alternative strategy to identify NIDDM loci based on linkage analysis (positional search) feasible. This strategy was used successfully in recent studies of IDDM (4) and in mapping genes for insulin resistance and insulin secretion in Pima Indians (1,5).

Chromosome 11 is particularly fertile ground for a positional search. Among the potential NIDDM candidates are two putative IDDM susceptibility loci (4,6), the recently identified familial hyperinsulinism locus (7), now thought to be the sulfonylurea receptor (8), and the apolipoprotein (apo) C3 cluster (apo C3, apo A1, and apo A4), which may cause insulin resistance through altered lipoprotein metabolism. Furthermore, both ataxia telangiectasia (11q telomeric to apo C3), which has been associated with marked insulin resistance (9), and muscle glycogen phosphorylase (PYGM), which may contribute to impaired muscle glycogen metabolism, have been mapped to chromosome 11.

We tested the hypotheses of linkage to these regions with closely spaced (<15 cM) microsatellite (dinucleotide repeat) markers under both model-dependent (parametric) methods and model-independent (nonparametric) methods. We provide some evidence for a possible NIDDM susceptibility locus in a region of <45 cM, which is near the IDDM4 locus. The best evidence is for a locus near marker D11S935, which does not fit our simple Mendelian models of inheritance.

RESEARCH DESIGN AND METHODS

Study population. All families were ascertained for a minimum of a sibling pair with NIDDM from a uniform population of northern European extraction, as described previously (10). Initial linkage studies were conducted on 346 members of 16 families or 405 family members from 19 families. Markers that were suggestive of possible linkage or that were particularly strong candidates were initially expanded into 10 additional families (136 individuals). Subsequently, the most positive markers were tested in an additional 16 families (82 individuals), of

From the Division of Endocrinology, Metabolism, and Diabetes (S.C.E., K.L.B., M.D.H., R.A.M.), Department of Veterans Affairs Medical Center and University of Utah; and the Department of Human Genetics (M.F.L.), Howard Hughes Medical Institute, University of Utah School of Medicine, Salt Lake City, Utah.

Address correspondence and reprint requests to Dr. Steven C. Elbein, Associate Professor of Medicine, Division of Endocrinology, Metabolism, and Diabetes, Endocrinology 111E, Department of Veterans Affairs Medical Center, 500 Foothill Blvd., Salt Lake City, UT 84148. E-mail: elbein@msscc.med.utah.edu.

Received for publication 27 June 1995 and accepted in revised form 2 November 1995.

APM, affected pedigree member; apo, apolipoprotein; LOD, logarithm of odds; NHANES, National Health and Nutrition Examination Survey; PYGM, muscle glycogen phosphorylase.

TABLE 1
Chromosome 11 markers for analysis

Locus	Dist	No. alleles	Het	Annealing temperature, °C	Cosol	Reference
D11S922	5	26	0.93	59	DMSO	3
TH	8	6	0.77	60	GLY	24
HBB	15	6	0.69	62/58	DMSO	25
D11S932	20	11	0.62	59	GLY	3
D11S861	26	12	0.86	56	DMSO	26
D11S902	29	11	0.83	59	DMSO	3
D11S929	40	10	0.84	59	DMSO	3
D11S935	52	8	0.83	59	DMSO	3
D11S907	54	—	0.71	57	DMSO	3
D11S905	62	12	0.83	59	DMSO	3
D11S987	75	14	0.84	59	DMSO	3
FGF3	82	10	0.77	55	DMSO	27
D11S1314	84	11	0.77	58	DMSO	3
D11S916	86	10	0.85	59	DMSO	3
D11S534	87	11	0.81	55	DMSO	28
D11S527	89	13	0.88	60	DMSO	29
D11S1321	90	7	0.63	58	DMSO	3
D11S901	97	70	0.81	59	DMSO	3
D11S923	113	11	0.81	59	DMSO	3
D11S927	120	11	0.86	59	DMSO	3
APOC3	134	24	0.95	67	GLY	30
D11S925	146	10	0.85	59	DMSO	3
D11S912	161	8	0.81	59	GLY	3
D11S968	177	9	0.81	59	DMSO	3

Loci for analysis of chromosome 11 are listed by map name. Dist is the cumulative distance from p-ter; Het is the heterozygosity calculated in our laboratory from unrelated spouses of family members; Cosol is the cosolvent used in the amplification, either 5% glycerol (GLY) or 5% DMSO. All primers were amplified in 1.5 mmol/l $MgCl_2$ except apo C3, which was amplified in 2.5 mmol/l $MgCl_2$.

which 13 families contained five or fewer available individuals. The latter two groups (26 families with 218 individuals) were considered an independent set for initial expanded studies but were ascertained under similar criteria to the screening set of pedigrees. At least 70 unrelated individuals were typed for allele frequencies. These individuals were spouses of nonfounding (second- and third-generation) family members or control individuals from other studies; some second-generation spouses were included in parametric analyses.

Individuals were considered affected if they were receiving medication for diabetes, had a fasting glucose level >7.8 mmol/l, or met age-specific 95% criteria for 2-h postchallenge glucose as approximated from the National Health and Nutrition Examination Survey (NHANES) dataset (11). Individuals were considered affected if the most recent postchallenge glucose level exceeded 7.8 mmol/l for individuals younger than age 45 years, 11.1 mmol/l for individuals between ages 45 and 64 years, or 13.3 mmol/l for individuals older than age 64. Uncertainty in diagnosis (extreme obesity, a single test with a postchallenge glucose value that did not exceed the above values by at least 20% or multiple tests with discrepant results) was reflected by liability class for parametric analysis (12). For nonparametric analysis, where uncertainty could not be included in the analysis, we chose a more conservative diagnostic scheme in which individuals with uncertain diagnosis were considered unaffected. These criteria were chosen in recognition of the substantial biological variability of postchallenge glucose values and laboratory variability in glucose measurements (GENNID Study Group, unpublished observations) and in an attempt to reduce the number of models tested to a minimum. The average number of individuals/family (excluding the small sibling pair families) was 22.5 (range 10–40), and the average number of affected individuals/family for parametric analysis was 6.1 (range 2–11). The maximum number of affected sibling pairs available for nonparametric analysis, including small sibling pair families, was 150.

Linkage analysis. Microsatellite markers were chosen from published maps and sequences (2,3,13) (Table 1) and were typed as described previously (10). Marker locations are shown in Fig. 1. Each marker was amplified using one γ-^{32}P–labeled primer from 100 ng of genomic DNA in 25 μl total volume. Each reaction contained 0.3–0.5 U of *Taq* polymerase, 10 pmol of each unlabeled primer, 0.5 pmol of labeled primer, and 200 μmol/l of each dideoxynucleotide. $MgCl_2$ concentration

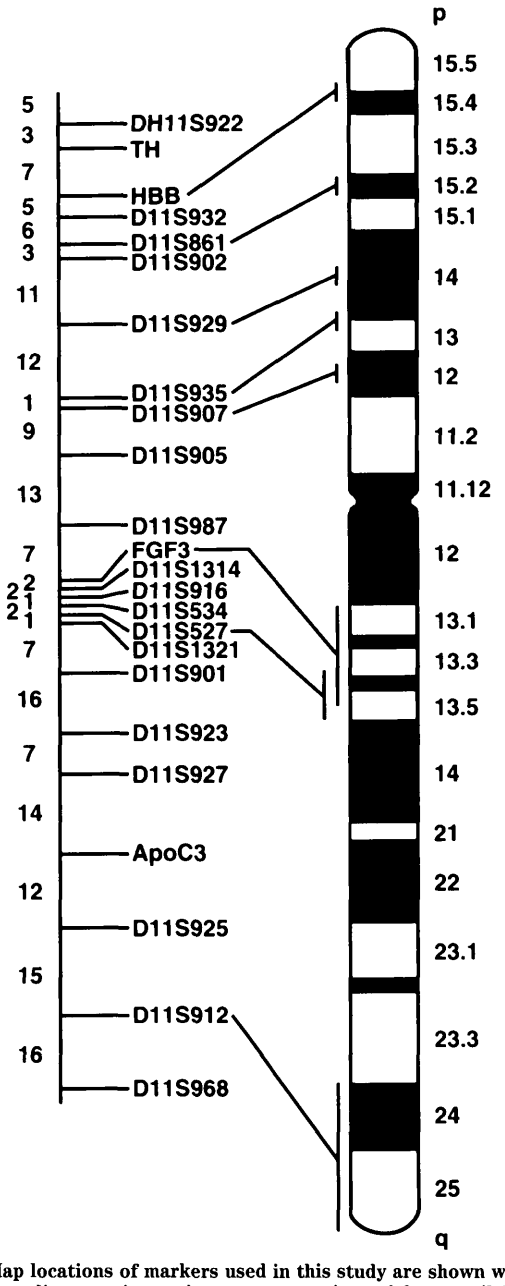

FIG. 1. Map locations of markers used in this study are shown with approximate distances in centimorgans, as estimated from available map data. Chromosomal locations and marker distances are based on data (2,3,13) and represent approximations.

is shown in Table 1. Reactions were performed in 96-well microtiter dishes in a Techne PHC-3 thermocycler (Techne, Princeton, NJ) and were loaded directly onto denaturing polyacrylamide gels from 96-well dishes with a multichannel pipet. All autoradiographs were scored by two readers with reference to pedigree structure but not affection status. Data from screening pedigrees were reexamined without reference to affection status, and ambiguous or absent typing was repeated before reanalysis.

Two-point parametric analyses were conducted with the MLINK program of the LINKAGE 5.1 package (14). Initial screening studies were performed with seven penetrance classes defined according to age and diagnosis and model parameters as described previously (10). Subsequently, we redefined model parameters to incorporate uncertainty of diagnosis and obesity. Thus, penetrance was defined for 10 liability classes, based on a linear age-specific penetrance estimated from epidemiological prevalence data (NHANES) (11) similar to that described previously (10) and as shown in Table 2. This penetrance was modified for BMI by adding an additional liability class for each 5 U of BMI >30 to a maximum of three liability classes (Table 2). Because no segregation analysis of NIDDM has provided convincing data for a single

TABLE 2
Liability classes for parametric analysis of NIDDM linkage data

Liability class	Age (years)	High-penetrance	High-penetrance sporadic	Low-penetrance	Low-penetrance sporadic
1	<30	0.15	0.001	0.05	0.001
2	30–40	0.30	0.005	0.10	0.005
3	40–50	0.45	0.01	0.15	0.010
4	50–60	0.60	0.02	0.20	0.015
5	60–70	0.75	0.03	0.25	0.020
6	>70	0.90	0.04	0.30	0.025
7	Obesity	0.90	0.05	0.30	0.025
8	Obesity	0.90	0.06	0.30	0.025
9	Obesity; uncertain diagnosis	0.90	0.08	0.30	0.030
10	Uncertain diagnosis	0.80	0.08	0.30	0.030

The table provides the penetrance function for parametric analysis using the LINKAGE 5.1 program. Actual liability class for linkage is adjusted upward for each 5 U of BMI >30 to a maximum of three classes. Individuals were placed in class 9 or 10 if diagnosis was based on the 2-h postchallenge glucose value from a single test or if BMI exceeded 45 and the diabetes did not require medication. The reduced penetrance model allowed for a higher percentage of nongenetic (sporadic) case for classes 1–9. Disease allele frequencies were set to 0.02 for dominant high-penetrance, 0.04 for dominant low-penetrance, and 0.20 for recessive high-penetrance models. With the sporadic frequency, these disease frequencies easily account for a 5–6% population prevalence among whites.

correct model and because testing of multiple models has been shown to provide an effective means to analyze data in which the true model is unknown (15), we tested three models: a dominant model with a maximum penetrance of 0.9; a recessive model with a maximum penetrance of 0.9; and a dominant model with a maximum penetrance of 0.3. All models assumed a 5% disease prevalence with disease allele frequencies under dominant, low-penetrance, and recessive models of 0.02, 0.04, and 0.20, respectively. Simulation studies have shown that small changes in these parameters do not significantly impact the logarithm of odds (LOD) score results (S.C. Elbein, unpublished observations). These models incorporate available age-specific incidence and prevalence data and the known influence of obesity for whites (11) and allow for maximum penetrance based on data from identical twins (16). We included an age-dependent phenocopy frequency, which allowed up to half of all NIDDM cases to be sporadic in older individuals (Table 2). Diagnostic uncertainty was reflected by a higher phenocopy ratio in liability classes 9 and 10. Nonparametric analyses were conducted with the affected pedigree member (APM) (17) programs using the intermediate weighting factor [$f(p) = 1/\sqrt{p}$] and with individuals in liability classes 9 and 10 considered unaffected. Individuals with IDDM were considered unknown, and allele frequencies were estimated from the maximum available number of unrelated individuals and nonfounding spouses (70–95 individuals). In all cases, analysis of markers expanded into new families was based on reestimated allele frequencies from additional unrelated individuals and the most recent diagnostic information.

Heterogeneity was formally tested under the most probable model for the combined pedigree sets using the admixture model as implemented in the HOMOG program (14). D11S902 was typed in all families despite initial lack of linkage.

Haplotype analysis. Haplotypes were constructed for 29 two- and three-generation families for markers D11S916, D11S534, D11S1321, and D11S527. These markers were confirmed to be closely linked on two-point linkage analysis with MLINK in disease pedigrees. Phase was established by descent, and rare ambiguous haplotypes were discarded from the analysis. Analysis was performed for identity by descent as described in Terwilliger and Ott (12).

RESULTS

Initial analyses of 19 closely spaced (<15 cM) markers (Fig. 1 and Table 2) in 16–19 moderately large Utah families are shown in Table 3. Of the families, 16 have been reported previously (10); three families added during the course of these studies were included for most markers. We found no evidence for linkage under either parametric or nonparametric analysis at any of the candidate regions: D11S922 and tyrosine hydroxylase, which are linked to IDDM2 (4); D11S902, which is closely linked to familial hyperinsulinism and the sulfonylurea receptor (7,8); and the apo C3 cluster or the ataxia telangiectasia locus, which maps between D11S925 and D11S912. Because the sulfonylurea receptor, which is near D11S902, represents a particularly strong candidate, we expanded D11S902 to an additional 10 families. We still found no evidence for linkage on parametric or nonparametric analysis (Table 4).

In contrast with findings at chromosome 11 candidate loci, some evidence for linkage was found in the broad region covering ~45 cM between D11S935 and D11S901 in studies conducted using nonparametric and parametric models with our previously reported liability function (seven liability classes based only on age). D11S935, D11S905, and D11S901 all show some evidence for linkage to NIDDM on nonparametric (APM) analysis, while maximum LOD scores for

TABLE 3
Results of screening studies of chromosome 11 markers

Marker	LOD, dominant	LOD, low-penetrance	LOD, recessive	APM, *P* value
D11S922	−9.52	−4.08	−10.14	0.42
TH	−7.32	−1.8	−6.34	0.69
HBB	−1.15	−2.18	−3.29 (0.37)	0.052
D11S932	−4.42	−1.44	−2.67 (0.46)	0.20
D11S861	−6.47	−2.94	−4.20	0.62
D11S902	−9.85	−3.52	−5.62	0.97
D11S929	−8.14	−1.84 (0.30)	−3.98 (0.58)	0.35
D11S935	−4.49	−0.54 (0.22)	−4.82	<0.0005
D11S905	−5.41	−0.62 (0.25)	−6.89	0.033
D11S987	−6.25	−0.94 (0.52)	−5.80	0.533
D11S916	−0.23*	2.06†	−2.08 (0.73)	<0.05
D11S527	−3.79 (0.31)	0.69‡	−6.03	0.44
D11S901	−4.16	−0.78 (0.29)	−2.44 (0.41)	<0.01
D11S923	−2.93 (0.31)	−0.37 (0.43)	−5.40	0.153
D11S927	−6.36 (0.23)	−3.41	−7.77	0.8
APOC3	−6.81 (0.47)	−3.13	−9.13	0.5
D11S925	−7.15	−2.79	−6.33	0.74
D11S912	−5.77	−2.02	−4.77	0.276
D11S968	−7.69	−1.59 (0.33)	−4.57	0.36

LOD scores and *P* values for APM analysis are reported for 19 families using previously described models with seven liability classes. LOD scores are reported at $\theta = 0.01$; *P* values are the $1/\sqrt{p}$ weighting factor. Low *P* values were also tested by simulation with 1,000–5,000 replications, and the significance based on simulation is reported. For D11S932, D11S861, D11S923, and D11S925, only 16 families were tested. Maximum LOD scores of $Z > 0.20$ are shown in parentheses; all values of θ were between 0.2 and 0.4 (not shown). *Maximum LOD score 1.63 at $\theta = 0.20$; †maximum LOD score 2.30 at $\theta = 0.05$; ‡maximum LOD score of 1.21 at $\theta = 0.10$.

TABLE 4
Expanded chromosome 11 markers

Marker	LOD, θ = 0.01, dominant low-penetrance	Maximum LOD, dominant low-penetrance	θ for maximum LOD	*P* value, APM analysis
D11S902	−4.79	0	0.50	0.982
D11S935	−1.19	0.12	0.20	0.00057 (0.0013)
D11S905	−2.44	0	0.50	0.300
D11S987	−2.95	0.03	0.30	0.283
FGF3	−3.15	−0.08	0.40	0.533
D11S1314	−1.94	0.10	0.30	0.582
D11S916	−1.09	0.91	0.10	0.039 (0.049)
D11S534	0.40	1.20	0.10	0.327
D11S527	−2.16	0.10	0.20	0.129
D11S1321	−0.85	0.02	0.30	0.072 (0.070)
D11S901	−2.32	0	0.50	0.041 (0.047)

Data are shown for markers expanded into the additional families for all available data. Markers D11S902, D11S987, FGF3, D11S1314, and D11S1321 were expanded only into 29 total families. Only the dominant low-penetrance parametric model is presented. Nonparametric analysis used the APM programs with the $1/\sqrt{p}$ allele weighting factor. Significance for APM analyses is shown for the reported asymptotic value; the value based on simulation with 5,000 replications is given in parentheses.

closely spaced markers D11S916 and D11S527 were 2.3 (θ = 0.05) and 1.21 (θ = 0.10), respectively, under a dominant reduced-penetrance model with seven age-dependent liability classes (Table 3). Based on these results, we tested linkage under the reduced penetrance and APM models for additional markers near D11S916: FGF3, D11S534, D11S1314, and D11S1321. No linkage was evident for FGF3, D11S1314, or D11S1321, but linkage of NIDDM to D11S534 showed a maximum LOD score of 2.01 at θ = 0.10.

We subsequently developed new parametric models, which modified the previous age-dependent liability by incorporating obesity (Table 2 and METHODS). Furthermore, the new models allowed for uncertainty in the diagnosis of individuals with extreme obesity or diagnosis based on a single glucose tolerance test. The original pedigree set was first reanalyzed for markers in the linked region based on these new models using diagnostic status updated from the most recent test results. For this analysis, we attempted to type samples that had previously failed typing. Allele frequencies were recalculated using additional unrelated individuals. Reanalysis resulted in somewhat lower maximum LOD scores for analysis of markers D11S916, D11S534, and D11S527 in the original 19-family set (1.646 at θ = 0.08; 1.837 at θ = 0.07; and 0.785 at θ = 0.12, respectively).

To further examine this region, we tested the 10 markers between D11S935 and D11S901 (inclusive) in 10 additional available families under the dominant low-penetrance model. The positive markers were subsequently also typed in 16 newly available families (3 multigenerational pedigrees and 13 sibling pair families consisting of one generation). Analysis of these families as a separate independent population did not support linkage for any marker (LOD scores between −2.5 and −1.0 at θ = 0.01, maxima of 0 at θ = 0.5). However, since both groups of pedigrees were ascertained similarly, we also tested the entire pedigree set for linkage under the dominant low-penetrance model and using APM analysis. The combined results for all available families are shown in Table 4. As expected, LOD scores for the combined population dropped further for markers D11S916, D11S527, and D11S534. LOD scores for all 45 families available for study are presented in Table 4 and were not significantly different from analysis of 29 multigenerational families. In contrast, APM analysis for markers D11S935, D11S916, and D11S901 was more significant for the 29-family set (simulated *P* values of 0, 0.018, and 0.014, respectively) and dropped somewhat when the more recently ascertained multigenerational families and 10 sibships were included (Table 4). We attempted to confirm linkage in the region of D11S935 using the marker D11S907, which is within 1 cM of D11S935 on the Généthon map (3) but 5 cM distant by MLINK analysis in our NIDDM families. Both parametric and APM analysis failed to support linkage for this marker (data not shown).

Multipoint APM analysis of four closely linked markers near IDDM4 (D11S916, D11S534, D11S1321, and D11S527) in 29 families was somewhat significant ($P = 0.01$), but sharing of haplotypes identical by descent constructed from these four markers for 129 affected sibling pairs did not deviate significantly from chance ($\chi^2 = 1.833$; $P = 0.18$). Furthermore, multipoint APM analysis of markers D11S916, D11S534, and D11S527 in the 45 families, which included several single-generation sibling pairs, failed to reach significance ($P = 0.07$). Finally, we examined markers D11S916, D11S527, D11S534, and D11S935 for evidence of a subgroup of families linked to NIDDM (admixture test) but were unable to formally demonstrate heterogeneity.

DISCUSSION

We tested 23 markers for linkage with familial NIDDM in a cohort of families ascertained for at least two NIDDM siblings and for grandparents of northern European descent. Several important candidate regions, including the familial hyperinsulinism/sulfonylurea receptor locus, were not linked to NIDDM under either parametric (LOD score) or nonparametric analyses. Because the sulfonylurea receptor is a particularly strong candidate, we tested the closely linked D11S902 marker in the 29-pedigree set but still found no evidence for linkage. We did, however, find some evidence for a susceptibility region near D11S935 under nonparametric (APM) analysis, although not on parametric analysis. Initial support for linkage at D11S905 locus (10 cM distant) was not confirmed in expanded studies. The only obvious candidate in this general region, PYGM, maps between D11S987 and D11S905 (2).

Because of the distance between D11S935 and D11S916, these regions are unlikely to represent a single susceptibility locus. In the full sample, evidence for linkage near D11S916 (IDDM4) is marginal without correction for multiple analytical methods or consideration of the real false-positive rate at this significance level for a whole genome search (see below). Nonetheless, several factors argue for evaluation in additional families. First, D11S916 is close to an IDDM4 locus for which linkage with IDDM has been replicated (4,6,18). IDDM loci that do not modulate the immune component of this disease must be considered strong candidates for NIDDM. Additionally, recent mapping of a mouse model of diet-induced NIDDM identified two susceptibility regions on mouse chromosome 7, one of which is syntenic with this general region of human chromosome 11 (19). These findings would support a diabetes susceptibility locus in this region,

albeit perhaps a weak one, which may require large numbers of families to duplicate.

Several factors suggest that our findings may represent spurious linkage. Parametric and nonparametric analyses gave different results. While this discrepancy may have an explanation (different diagnostic criteria, different analytical power), concordance among methods would increase our confidence that the linkage was real. Second, closely linked markers did not confirm the original observations. Finally, expanding the sample size did not improve the significance.

Traditionally, significance for parametric analysis requires a LOD score ≥3.0. Correction for testing three parametric models would raise this level to 3.5 (12), and additional correction for a single nonparametric analysis would raise this higher. Only our APM analysis of D11S935 approached these criteria ($P < 0.0001$ by asymptotic estimate in 29 families, although only 0.001 by simulation in 45 families). Thus, with significance estimated by simulation for all available pedigrees and sibships, no marker achieved the required level of significance of 10^{-4} even without correction for multiple analyses. Kruglyak and Lander (20) recently calculated that for a whole genome search, a false-positive rate of 5% corresponded to a LOD score of 4 or a P value of 0.00002. Our results, which do not approach these levels, thus have substantial potential to represent a false-positive result.

Several investigators have noted the difficulty in achieving even the traditional level of significance in the face of uncertainties about diagnosis, a high sporadic frequency, and probable heterogeneity (21). These cautions seem to be confirmed in the initial linkage studies of IDDM, in which only the human leukocyte antigen region met even traditional criteria (4). No other locus approached the criteria suggested by Kruglyak and Lander, despite apparent independent replication of linkage for 10 numbered loci (22). These investigators also failed to replicate their findings in one or more populations of comparable genetic background. Even in apparently monogenic diseases, linkage has been rejected at a locus adjacent to significantly positive findings (23). Consequently, only the attempt to replicate our findings in several independent NIDDM populations, particularly those of northern European extraction, will determine the true significance of this region as an NIDDM susceptibility locus. Alternatively, when the specific IDDM4 gene is cloned or additional candidate loci near D11S935 are located, we can search for mutations among NIDDM individuals, particularly those individuals belonging to families with positive LOD scores.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant DK-39311 and by the Research Service of the Department of Veterans Affairs. Pedigree sampling and phenotypic analysis were also supported by Public Health Service Grant M01-RR00064 from the National Center for Research Resources to the General Clinical Research Center and in part by a Harold Rifkin family acquisition grant from the American Diabetes Association. Primer synthesis was supported by Public Health Service (GESTEC) Grant 5-P30-HG00199 (to M.L.).

We thank M. Alan Permutt for sharing information on the familial hyperinsulinism locus and Kim Wegner, Cindy Miles, and Teresa Maxwell for excellent assistance in pedigree ascertainment and sampling and DNA preparation.

REFERENCES

1. Elbein SC, Hoffman MD, Bragg KL, Mayorga RA: The genetics of NIDDM. *Diabetes Care* 17:1523–1533, 1994
2. Cooperative Human Linkage Center: A comprehensive human linkage map with centimorgan density. *Science* 265:2049–2054, 1994
3. Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J: The 1993–94 Généthon human genetic linkage map. *Nat Genet* 7:246–339, 1994
4. Davies JL, Kawaguchi Y, Bennet ST, Copeman JB, Cordell HJ, Pritchard LE, Reed PW, Gough SCL, Jenkins SC, Palmer SM, Balfour KM, Rowe BR, Farrall M, Barnett AH, Bain SC, Todd JA: A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 371:130–136, 1994
5. Thompson DB, Janssen RC, Ossowski VM, Prochazka M, Knowler WC, Bogardus C: Evidence for linkage between a region on chromosome 1p and the acute insulin response in Pima Indians. *Diabetes* 44:478–481, 1995
6. Field LL, Tobias R, Magnus T: A locus on chromosome 15q26 (IDDM3) produces susceptibility to insulin-dependent diabetes mellitus. *Nat Genet* 8:189–194, 1994
7. Glaser B, Chiu KC, Anker R, Nestorowicz A, Landau H, Ben-Bassat H, Shlomai Z, Kaiser N, Thornton PS, Stanley CA, Spielman RS, Gogolin-Ewens K, Cerasi E, Baker L, Rice J, Donis-Keller H, Permutt MA: Familial hyperinsulinism maps to chromosome 11p14–15.1, 30 cM centromeric to the insulin gene. *Nat Genet* 7:185–188, 1994
8. Thomas PM, Cote GJ, Wohilk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J: Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 268:426–429, 1995
9. Gatti RA, Berkel I, Boder E, Braedt G, Charmley P, Concannon P, Ersoy F, Foroud T, Jaspers NGJ, Lange K, Lathrop GM, Leppert M, Nakamura Y, O'Connell P, Paterson M, Salser W, Sanal O, Silver J, Sparkes RS, Susi E, Weeks DE, Wei S, White R, Yoder F: Localization of an ataxia-telangiectasia gene to chromosome 11q22–23. *Nature* 336:577–580, 1988
10. Elbein SC, Hoffman M, Ridinger D, Otterud B, Leppert M: Description of a second microsatellite marker and linkage analysis of the muscle glycogen synthase locus in familial non-insulin dependent diabetes. *Diabetes* 43:1061–1065, 1994
11. National Diabetes Data Group: *Diabetes in America.* Harris MI, Hamman RF, Eds. Bethesda, MD, U.S. Dept. of Health and Human Services, 1985 (NIH publ. no. 85–1468)
12. Terwilliger JD, Ott J: *Handbook of Human Genetic Linkage.* Baltimore, MD, Johns Hopkins Univ. Press, 1994
13. Litt M, Kramer P, Hauge XY, Weber JL, Wilkie PJ, Holt MS, Mishra S, Donnis-Keller H, Warnich L, Retief AE, Jones C, Weissenbach J: A microsatellite-based index map of human chromosome 11. *Hum Mol Genet* 2:909–913, 1993
14. Ott J: *Analysis of Human Genetic Linkage.* Revised ed. Baltimore, MD, Johns Hopkins Univ. Press, 1991
15. Cox NJ, Hodge SE, Marazita ML, Spence MA, Kidd KK: Some effects of selection strategies on linkage analysis. *Genet Epidemiol* 5:289–297, 1988
16. Barnett AH, Eff C, Leslie RDG, Pyke DA: Diabetes in identical twins: a study of 200 pairs. *Diabetologia* 87–93, 1981
17. Weeks DE, Lange K: The affected-pedigree-member method of linkage analysis. *Am J Hum Genet* 42:315–326, 1988
18. Hashimoto L, Habita C, Beressl JP, Delepine M, Besse C, Cambon-Thomsen A, Deschamps I, Rotter JI, Djoulah S, James MR, Froguel P, Weissenbach J, Lathrop GM, Julier C: Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. *Nature* 371:161–164, 1994
19. Seldin MF, Mott D, Bhat D, Petro A, Kuhn CM, Kingsmore SF, Bogardus C, Opara E, Feinglos MN, Surwit RS: Glycogen synthase: a putative locus for diet-induced hyperglycemia. *J Clin Invest* 94:269–276, 1994
20. Kruglyak L, Lander ES: Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57:439–454, 1995
21. Thompson G: Identifying complex disease genes: progress and paradigms. *Nat Genet* 8:108–110, 1994
22. Todd JA: Genetic analysis of type 1 diabetes using whole genome approaches. *Proc Natl Acad Sci USA* 92:8560–8565, 1995
23. Jamieson CR, van der Burgt I, Brady AF, Reen MV, Elsawi MM, Hol F, Jeffery S, Patton MA, Mariman E: Mapping a gene for Noonan syndrome to the long arm of chromosome 12. *Nat Genet* 8:357–360, 1994
24. Polymeropoulos MH, Xiao H, Rath DS, Merril CR: Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). *Nucl Acid Res* 19:3753, 1991
25. Hauge XY, Litt R, Litt M: Dinucleotide repeat polymorphisms at the D11S439 and HBB loci. *Hum Mol Genet* 1:548, 1992
26. Hudson TJ, Engelstein M, Lee MK, Ho EC, Rubenfield MJ, Adams CP, Houseman DE, Dracopoli NC: Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. *Genomics* 13:622–629, 1992
27. Polymeropoulos MH, Xiao H, Rath DS, Merril CR: Dinucleotide repeat

polymorphism at the int-2 protooncongene locus (INT2). *Nucl Acid Res* 18:7468, 1990
28. Haugue XY, Evans GA, Litt M: A dinucleotide repeat polymorphism at D11S534. *Nucl Acid Res* 19:4308, 1991
29. Browne DL, Gault J, Thompson MB, Hauge XY, Evans GA, Litt M: Dinucleotide repeat polymorphism at the D11S527 locus. *Nucl Acid Res* 19:4790, 1991
30. Bhattacharya S, Wilson TME, Wojciechowski AP, Volpe CP, Scott J: Hypervariable polymorphism in the APOC3 gene. *Nucl Acid Res* 19:4799, 1991