Am. J. Hum. Genet. 64:1127–1140, 1999

Linkage of Type 2 Diabetes Mellitus and of Age at Onset to a Genetic Location on Chromosome 10q in Mexican Americans

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Summary

Since little is known about chromosomal locations harboring type 2 diabetes–susceptibility genes, we conducted a genomewide scan for such genes in a Mexican American population. We used data from 27 low-income extended Mexican American pedigrees consisting of 440 individuals for whom genotypic data are available for 379 markers. We used a variance-components technique to conduct multipoint linkage analyses for two phenotypes: type 2 diabetes (a discrete trait) and age at onset of diabetes (a truncated quantitative trait). For the multipoint analyses, a subset of 295 markers was selected on the basis of optimal spacing and informativeness. We found significant evidence that a susceptibility locus near the marker D10S587 on chromosome 10q influences age at onset of diabetes (LOD score 3.75) and is also linked with type 2 diabetes itself (LOD score 2.88). This susceptibility locus explains 63.8% ± 9.9% (P = .000016) of the total phenotypic variation in age at onset of diabetes and 65.7% ± 10.9% (P = .000135) of the total variation in liability to type 2 diabetes. Weaker evidence was found for linkage of diabetes and of age at onset to regions on chromosomes 3p, 4q, and 9p. In conclusion, our strongest evidence for linkage to both age at onset of diabetes and type 2 diabetes itself in the Mexican American population was for a region on chromosome 10q.

Received August 3, 1998; accepted for publication January 28, 1999; electronically published March 12, 1999.

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0002-9297/99/6404-0025$02.00

Introduction

Type 2 diabetes mellitus is a complex blood glucose–homeostasis disorder characterized by both insulin resistance and pancreatic β-cell dysfunction (Rewers and Hamman 1995; Polonsky et al. 1996). The belief that type 2 diabetes has strong genetic determinants is based on several lines of evidence, including the high rate of concordance among MZ twins (Barnett et al. 1981; Newman et al. 1987), the marked disease-rate differences between populations (Zimmet 1979; Diehl and Stern 1989; McKeigue et al. 1991), and the close correspondence, in hybrid populations, between genetic admixture rates and disease prevalence (Brosseau et al. 1979; Chakraborty et al. 1986; Knowler et al. 1988). In addition, there is evidence for major gene(s) influencing diabetes or its related phenotypes, such as glucose concentrations, 2-h insulin levels, and age at onset of diabetes (Elston et al. 1974; Serjeantson and Zimmet 1984; Hanson et al. 1995; Mitchell et al. 1995; Stern et al. 1996*b*). However, the mode of inheritance for type 2 diabetes appears to be variable across populations, suggesting a complex genetic architecture underlying this disease.

Since a number of genes are involved in the pathways of insulin action and glucose-stimulated insulin secretion, any genetic defect in these pathways might contribute to type 2 diabetes (Pillay et al. 1995; Kahn et al. 1996). Numerous studies are in progress that are examining the possible roles of such candidate genes in diabetes. However, the genes thus far examined appear to account for a very small proportion of the susceptibility to type 2 diabetes, and significant findings in some populations often fail to be replicated in others (Elbein 1997). It is unclear to what extent inconsistent results across populations could be due to factors such as different study designs (sib pairs, extended pedigrees, etc.), sample size, choice or power of the analytical methods (affected-sib-pair–based methods, variance-components approaches, etc.) or to genuine heterogeneity of the disease itself (McCarthy et al. 1994; Ghosh and Schork 1996; Elbein 1997).

Table 1

Numbers and Types of Relative Pairs in a Subset of SAFADS Families in the Present Study, Including 440 Individuals Distributed across 27 Pedigrees

Type of Relative Pair	Relationship Coefficient[a]	No. of U-U	No. of U-A	No. of A-A
Parent-offspring	.5000	184	219	52
Sibs	.5000	334	150	49
Grandparent-grandchild	.2500	48	76	4
Avuncular[b]	.2500	324	322	44
Half-sibs	.2500	53	17	6
Great-grandparent–grandchild	.1250	12	1	...
Grand avuncular	.1250	87	63	1
Half-avuncular	.1250	22	49	3
First-cousins	.1250	471	201	16
Great–grand avuncular	.0625	3	...	...
Half–grand avuncular	.0625	9	2	...
First cousins, once removed	.0625	407	118	...
Half–first cousins	.0625	62	31	...
First cousins, twice removed	.0312	9	...	...
Half–first cousins, once removed	.0312	33	2	...
Second cousins	.0312	213	33	...
Second cousins, once removed	.0156	15	...	...
Total		2,286	1,284	175

[a] $2 \times \Phi_{ij}$.

[b] Includes all aunt/uncle-niece/nephew combinations.

The Mexican American population, a genetically admixed group with both Native American and white ancestry, exhibits higher incidence of type 2 diabetes than is seen in the general population of the United States, and this excess incidence is thought to be related to their Native American ancestry (Weiss et al. 1984; Diehl and Stern 1989). In the present study, we utilized a multipoint variance-components approach to scan the genome for type 2 diabetes–susceptibility genes in a low-income Mexican American population, using data from 440 individuals distributed across 27 extended pedigrees. This method is less penetrance-model dependent than the classical segregation/linkage-mapping technique. Our genomewide scan for type 2 diabetes–susceptibility genes used two phenotypes: type 2 diabetes (a dichotomous trait) and age at onset of type 2 diabetes (a truncated quantitative trait). Genotypic information from 379 microsatellite markers across the genome was used in the linkage analyses.

Subjects and Methods

Subjects

Probands for the San Antonio Family Diabetes Study (SAFADS) were type 2 diabetics identified in an earlier epidemiological survey, the San Antonio Heart Study, which has been extensively described in previous publications (Stern et al. 1984; Stern and Haffner 1990). In brief, only low-income Mexican Americans identified in the San Antonio Heart Study as having type 2 diabetes were eligible to be probands. All first-, second-, and third-degree relatives, ages 18–98 years, were invited to participate in the study. In total, 579 individuals (140 diabetics) were examined, who were distributed across 32 extended families. The family-size range was 2–53 individuals (median family size 14 individuals). Metabolic, anthropometric, and demographic data plus medical histories were obtained on all the examined individuals. All procedures were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and all subjects gave informed consent.

Blood samples were obtained after a 12-h fast for assessment of various metabolic traits, including plasma glucose levels. Glucose levels were also measured 2 h after a standardized oral glucose load. Glucose levels were measured by means of an Abbott Biochromatic Analyzer. Diabetes was diagnosed according to World Health Organization criteria (World Health Organization Expert Committee 1985). Subjects who did not meet these criteria but who gave a history of diabetes and who reported that they were under treatment with oral antidiabetic agents or insulin were also considered to have diabetes.

A systematic genomewide search for type 2 diabetes–susceptibility genes was performed on a subset of 440 participants in the 27 most informative extended families. Of the 440 individuals, 116 are diabetics (including probands), giving a prevalence of 26.4%. There are 3,745 relative pairs, with varying degrees of genetic

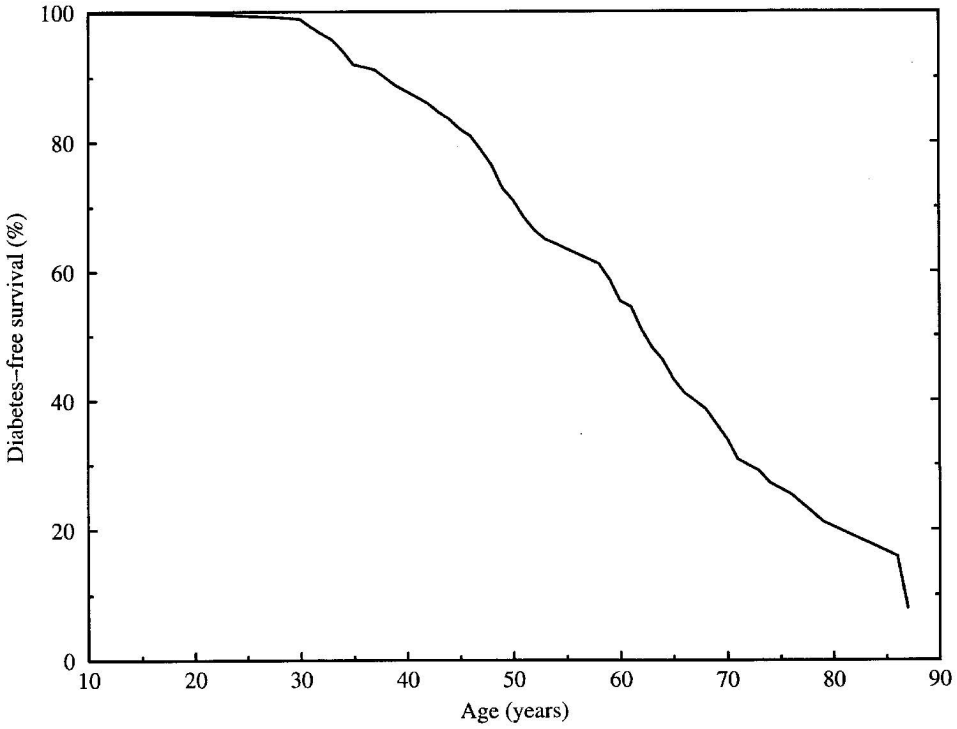


Figure 1 Diabetes-free survival among the SAFADS participants distributed across 27 pedigrees

relationship (table 1). Of these pairs, 2,286 are unaffected-unaffected (U-U) pairs, 1,284 are unaffected-affected pairs (U-A), and 175 are affected-affected pairs (A-A). When parent-offspring pairs (which are uninformative with respect to linkage) are excluded, the distribution of the three categories of relative pairs is as follows: U-U 2,102; U-A 1,065; and A-A 123.

Of the 116 diabetics, 50 subjects (43%) were clinically diagnosed at the time of their SAFADS examination. The remaining 66 diabetics (57%) were diagnosed prior to the study, and their age-at-onset information was based on self-reporting. The mean age at onset for the 116 affecteds was 49.8 ± 14.1 years. The mean age at onset for the subjects diagnosed at the time of their SAFADS examination was 53.1 ± 16.2 years, compared with 47.3 ± 11.8 years for subjects diagnosed prior to the study. The mean age of unaffecteds was 38.3 ± 15.4 years. Figure 1 depicts the diabetes-free survival among the SAFADS participants, as based on the Kaplan-Meier method as implemented in the SAS computer program (Cantor 1997).

Genotyping

The identity of each marker tested and the LOD scores (both two-point and multipoint) estimated can be found at our Website (MARS). For the multipoint analyses, markers with <~80% of the sample genotyped were not used unless their absence would result in a gap of ⩾20 cM. Thus, a subset of 295 markers was selected on the basis of optimal spacing and informativeness. Primers were purchased from Research Genetics. Further details concerning oligonucleotide-primer sequences and polymorphisms can be obtained from the Genome Database at Johns Hopkins University (Fasman et al. 1996). PCR conditions were optimized by testing a range of annealing temperatures with varying concentrations of $MgCl_2$ (1.0, 1.5, 2.0, 2.5, 3.0, 3.5 or 4.0 mM), so that the PCR amplification produced a specific product that could be visualized by ethidium bromide staining (0.5 μg/ml).

Genotypes were collected primarily by PCR assays with radiolabeled oligonucleotide primers. The antisense primer was 5′-radiolabeled, in a standard polynucleotide kinase reaction, with 3,000 Ci/mmol [γ^{32}P]-ATP (NEN) at a molar ratio of 18 [γ^{32}P]-ATP:1 primer, as described elsewhere (O'Connell et al. 1995; Duggirala et al. 1996). Thirty cycles of PCR (denaturing at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 1 min) were performed in a 15-μl assay containing 50 ng of DNA template, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1–4 mM $MgCl_2$, 0.1 M spermidine (Sigma) 0.1 μM of each PCR primer, 0.1 μM of each dNTP (Gibco BRL), and 0.5 units of *Taq* polymerase (Perkin Elmer). Amplified DNA was diluted 1:1 with stop solution (97%

formamide, 1% EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol) and was denatured at 85°C for 2 min. Three microliters of denatured DNA from each sample were loaded onto a 7% denaturing polyacrylamide gel (19:1 acrylamide/bis-acrylamide) containing 32% formamide and 34% urea and were fractionated by gel electrophoresis for 2.5 h at 60 W. Gels were transferred to filter paper (Whatman 3MM; WR Balston) covered with plastic wrap, were equilibrated with 20% methanol/20% acetic acid solution, and were dried. Dried gels were exposed to X-ray film (Fuji Photo Film). Data for a subset (65) of the markers were collected by use of fluorescently labeled primers purchased from Research Genetics; these were PCR amplified as described above and were loaded onto an Applied Biosystems Model 373 sequencer, and the data were analyzed by Applied Biosystems GENOTYPER software.

The genotypic data were entered into a database and were analyzed for discrepancies (i.e., violations of Mendelian inheritance), by the program INFER (Dyke 1996). In the laboratory, discrepancies were checked for mistyping, and markers for discrepant individuals were either corrected or blanked out prior to analysis. Our genomewide screen currently provides coverage at 10–20-cM intervals on chromosomes 1–22.

Statistical Genetic Analyses

We used a multipoint variance-components procedure to test for linkage between marker loci and two phenotypes: type 2 diabetes (a dichotomous trait) and age at onset of diabetes (a quantitative trait). This technique, originally developed for quantitative traits (Blangero and Almasy 1997; Comuzzie et al. 1997), has recently been extended to dichotomous-trait linkage analysis using a threshold model (Duggirala et al. 1997). We have now modified this procedure to allow for truncated traits, such as age at onset.

The threshold model assumes that an individual belongs to a specific disease category if an underlying genetically determined risk or liability exceeds a certain threshold, T, on a normally distributed liability curve. The liability is assumed to have an underlying multivariate-normal distribution. The correlation between pairs of individuals is estimated on the basis of the affection status of unrelated individuals and various classes of relatives. For a simple model, the liability correlation (ρ) between individuals i and j is given by $\rho_{ij} = \pi_{mij}h_m^2 + 2\Phi_{ij}h^2 + I_{ij}e^2$, where π_{mij} is the proportion of genes that individuals i and j share identical by decent (IBD) at a marker, m, linked to a quantitative-trait locus (QTL); h_m^2 is the heritability attributed to the marker locus linked to a QTL; Φ_{ij} is the kinship coefficient for individuals i and j; h^2 is the heritability attributed to additive polygenic effects; I_{ij} is the coefficient for the random environmental component for individuals i and j, which equals 1 if $i = j$ and 0 for all $i \neq j$; and $e^2 = 1 - (h_m^2 + h^2)$. Since calculation of the likelihood for this multifactorial model requires high-dimensional integration, we evaluated it approximately, using the Mendell/Elston algorithm (Mendell and Elston 1974). The variance-components and covariate effects for type 2 diabetes (i.e., sex and sex-specific age effects) are estimated, simultaneously, by maximum-likelihood techniques.

In contrast to the threshold model described above, in which age is incorporated as a covariate, the age-at-onset analysis dissects the actual trait: age at onset of diabetes. In this model, we allow age at onset to be known or right-truncated in diabetics (i.e., in affected subjects, age at onset of diabetes is less than or equal to age at examination) and to be left-truncated in nondiabetics (i.e., in unaffected subjects, age at onset is greater than age at examination). It is assumed that all individuals are susceptible to type 2 diabetes and would ultimately be affected if they lived long enough. This is a reasonable assumption for common adult-onset diseases that are related to physiological dysfunction. In fact, the diabetes-free survival patterns among the SAFADS participants (fig. 1) justify this assumption. The age at onset (for the diabetics) and age at examination (for the nondiabetics) determine the interval of integration, thus leading to an integration problem very similar to that in the purely discrete-trait case.

For the age-at-onset analysis, the variance components are estimated together with the phenotypic mean, the phenotypic SD, and sex as a covariate. Since the SAFADS families were ascertained on the basis of type 2 diabetic probands, our analyses included ascertainment correction. We corrected for the ascertainment by conditioning on the likelihood of observing the diabetic condition of the proband (Cannings and Thompson 1977). The hypothesis tests are performed by likelihood-ratio tests. The hypothesis of no linkage is tested by comparison of the likelihood in a model in which the parameter h_m^2 is constrained to a value of 0 versus the likelihood in a model in which the same parameter is estimated. Twice the difference in ln likelihoods of these models yields a test statistic that is asymptotically distributed, approximating a χ^2 distribution (in fact, as a $\frac{1}{2}$:$\frac{1}{2}$ mixture of a χ_1^2 and a point mass at 0 [Self and Liang 1987]). The df are equal to the difference in the number of parameters estimated in the two competing models. LOD scores are obtained by conversion of the ln-likelihood values into values of log to the base 10.

Locus-specific IBD information for pairs of relatives in SAFADS families were obtained by an exact recursive algorithm (Davis et al. 1996), by the program IBDMAT. The multipoint mapping strategy proposed by Fulker et al. (1995) has been extended and modified (Blangero and Almasy 1997; Almasy and Blangero 1998) to per-

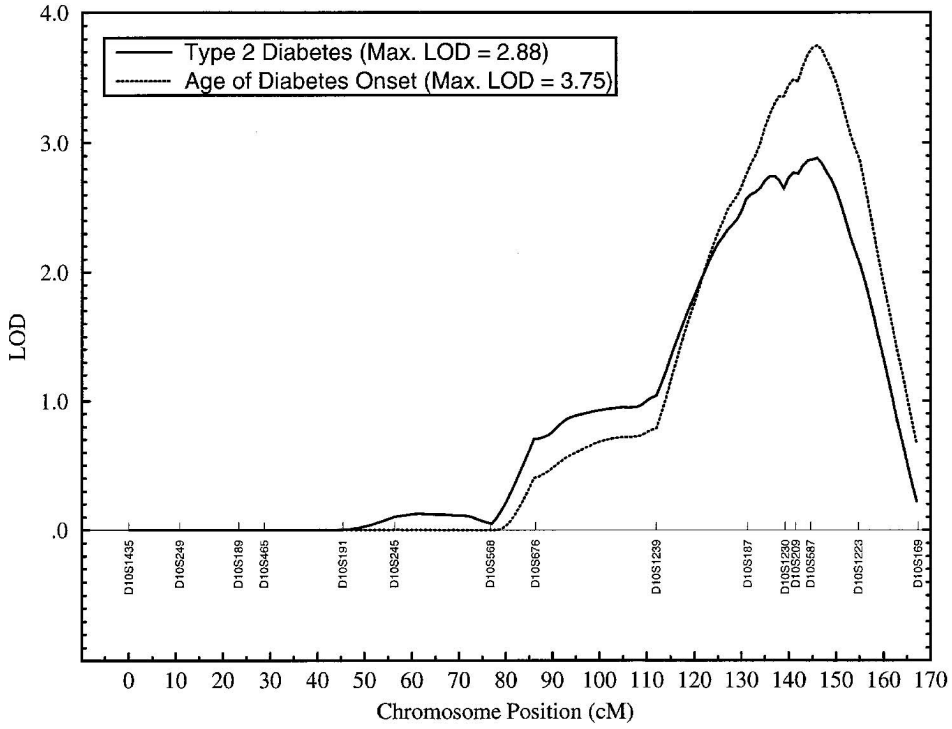


Figure 2 Linkage of type 2 diabetes and age at onset of diabetes to a genetic location near the microsatellite marker D10S587 on chromosome 10q.

form the multipoint variance-components analysis. Since the method requires the distances between the markers to be known, the order of loci spanning a given chromosome and the maximum-likelihood sex-averaged map distances between the marker loci were obtained by Kosambi's mapping function, by the program CRI-MAP (Green et al. 1990), supplemented by the map information in the Genome Database (Fasman et al. 1994, 1996) and Genetic Location Database (Collins et al. 1996). For each chromosome, a two-point variance-components method is applied at 1-cM intervals. The multipoint variance-components procedure for both discrete and truncated quantitative traits has been implemented in the computer program SOLAR (Almasy and Blangero 1998).

Results

Prior to the linkage analyses, for the both type 2 diabetes phenotype and the age at onset of type 2 diabetes phenotype, we first quantified the respective proportions of variance that were attributable to additive genetic factors (h^2). After correcting for the effects of sex and sex-specific age effects, we detected high heritability for type 2 diabetes (53.0% ± 17.2%), which was statistically highly significant ($P < .0001$). Individual specific random environmental factors account for 47.0% ± 17.2% of variation. The heritability for age at onset of diabetes was also high (45.5% ± 15.8%) and statistically significant ($P = .0001$), and random environmental effects contribute 54.5% ± 15.8% of total variation for this phenotype. Thus, additive genetic factors alone explain much of the phenotypic variation of both type 2 diabetes and age at onset of diabetes.

The linkage results reported here refer to the multipoint variance-components linkage analyses only, and all LOD scores are multipoint LOD scores. Overall, both phenotypes exhibited similar linkage profiles across the genome (Appendix). To determine whether our results are robust to transformation, we repeated the analyses, using log-transformed age-at-onset data. The resultant LOD scores were almost identical to those obtained from the untransformed data. We have elected to present the results by using the untransformed age-at-onset analyses in the present study. The highest LOD score was 3.75, between a genetic location near the marker D10S587 on chromosome 10 and age at onset of type 2 diabetes. This susceptibility locus, which is ~1 cM telomeric to the marker D10S587, explains 63.8% ± 9.9% ($P = .000016$) of the total phenotypic variation in age at onset of diabetes. The discrete trait, diabetes, is also linked to the same genetic location (LOD score 2.88), and this

Table 2

Peak Multipoint LOD Scores at Genetic Locations with the Nearest Flanking Markers on Chromosomes 3, 4, 9, and 10

Marker Region	Map Position (cM)	Peak Multipoint LOD Score for[a]	
		Type 2 Diabetes	Age at Onset of Diabetes
Chromosome 3:			
D3S1566	59	...	...
Peak	70/71	2.67 (71 cM)	2.51 (70 cM)
GATA128C02	75	2.56	2.29
Chromosome 4:			
D4S1615	170	1.94	...
Peak	172	1.99	...
D4S175	177	...	...
Chromosome 9:			
D9S288	0	...	...
Peak	14/16	2.38 (16 cM)	2.06 (14 cM)
D9S925	26	...	...
Chromosome 10:			
D10S587	145	2.87	3.73
Peak	146	2.88	3.75
D10S1223	155	2.07	2.87

[a] Only values ⩾1.9 are shown (as suggested by Lander and Kruglyak [1959]; see text).

putative locus accounts for 65.7% ± 10.9% (P = .000135) of the total variation in liability to type 2 diabetes. It should be noted that the trait-specific (i.e., diabetes and age at onset) additive genetic variance explained by this susceptibility locus is greater than the additive genetic variance attributable to polygenic effects. The likely explanation for this apparent anomaly is that the estimation of additive genetic variance attributable to polygenic effects may be biased when, as suggested by our data, major-gene effects are present.

The LOD scores obtained for both the diabetes analysis and the age-at-onset-of-diabetes analysis were plotted against map positions on chromosome 10 (fig. 2). As can be seen in figure 2, the two analyses yielded similar topologies of likelihood function, for localization of the susceptibility locus near the marker D10S587. The highest LOD scores for both phenotypes occur near the marker D10S587, but the respective peaks are relatively broad. Specifically, the three sequential markers D10S1230, D10S209, and D10S587 (which cover an ~8-cM region) in this chromosomal region exhibit relatively strong linkage with both phenotypes: age at onset of diabetes (LOD scores: D10S1230, 3.36; D10S209, 3.48; and D10S587, 3.73) and diabetes (LOD scores: D10S1230, 2.65; D10S209, 2.76; and D10S587, 2.87). Both phenotypes peak at the same genetic location (~1 cM telomeric to the marker D10S587), with the highest LOD scores (diabetes, 2.88; and age at onset of diabetes, 3.75) (table 2).

The following chromosomal regions (table 2) show suggestive linkage (i.e., multipoint LOD score ⩾1.9) to age at onset of diabetes: a genetic location near the marker GATA128C02, on chromosome 3 (LOD score 2.51), and a location on chromosome 9, between the markers D9S288 and D9S925 (LOD score 2.06). The genetic location on chromosome 3 (near the marker GATA128C02) is also statistically linked with type 2 diabetes, with a LOD score of 2.67. Two other chromosomal regions that have suggestive linkages with type 2 diabetes are a location between the markers D9S288 and D9S925 (LOD score 2.38) on chromosome 9 and a location between markers D4S1615 and D4S175 on chromosome 4 (LOD score 1.99). No other multipoint LOD scores ⩾1.9 were observed.

Discussion

Type 2 diabetes continues to be a leading cause of morbidity and mortality, especially in the developed countries. Despite the belief that type 2 diabetes is under genetic control and that it results from complex networks of gene(s) × environment interactions, little is known about the chromosomal locations harboring type 2 diabetes–susceptibility genes. In recent years, susceptibility genes for monogenic forms of type 2 diabetes (i.e., maturity-onset diabetes of the young [MODY]) have been successfully mapped and, in some cases, identified. MODY1 has been localized to a region on chromosome 20q (Bell et al. 1991); MODY2 has been linked to the glucokinase locus on chromosome 7p (Frougel et al. 1992); and MODY3 has been localized to a region on chromosome 12q (Vaxillaire et al. 1995). As reviewed by Elbein (1997), the genes responsible for these MODY variants have been identified as follows: hepatocyte nuclear-transcription factor 4α for MODY1, glucokinase for MODY2, and hepatocyte nuclear-transcription factor 1α for MODY3. The genetic basis of the common form of type 2 diabetes, however, continues to be elusive. In the present study, we found evidence for significant linkage of age at onset of diabetes to a chromosomal region on chromosome 10q, which appears to have substantial influence on both the age-at-onset-of-diabetes phenotype and the type 2 diabetes phenotype.

Using a "candidate gene approach," numerous studies have examined whether genes involved in the pathways of either insulin secretion or insulin action could influence susceptibility to the common form of type 2 diabetes. Thus far, however, none of these candidate gene has consistently been found to play a substantial role in the pathogenesis of type 2 diabetes (Elbein 1997). In part, inconsistencies across the studies may be due either to the heterogeneous nature of type 2 diabetes or to the limitations of the various strategies for detection of linkage (e.g., problems of model misspecification, which are

associated with classical segregation/linkage analysis) (McCarthy et al. 1994; Ghosh and Schork 1996).

Recently, two type 2 diabetes–susceptibility loci have been reported by two groups, using genomewide scans, who have designated them as "NIDDM1" (Hanis et al. 1996) and "NIDDM2" (Mahtani et al. 1996). Using the data on Mexican American affected sib pairs from Starr County, Texas, Hanis et al. (1996) found evidence for linkage of type 2 diabetes to the telomeric region of chromosome 2q (D2S125). However, this finding has not been replicated in other studies (Elbein 1997; Mahtani et al. 1996; McCarthy et al. 1997), including the present study. Likewise, Hani et al. (1997*b*) failed to find evidence for linkage between D2S125 and type 2 diabetes in French families; but they found some evidence for linkage between type 2 diabetes and the marker D2S140, which is located 3 cM from D2S125. However, their results became nonsignificant when a multipoint approach was used with weighting for nonindependent sib pairs.

Mahtani et al. (1996) reported linkage of an insulin-deficient form of type 2 diabetes to a location near marker D12S1349 on chromosome 12q. In fact, they were able to find evidence for linkage of this susceptibility locus, NIDDM2, to type 2 diabetes only in a subset (25%) of the original 26 families from the Bothnia region of western Finland. This chromosomal region contains the MODY3 locus. Given that NIDDM2 appears to be linked to a distinct form of type 2 diabetes associated with low insulin secretion, no evidence for linkage of this region to the common form of type 2 diabetes has been found, thus far, in other studies (Elbein 1997), including the present study. Recently, the report by Mahtani et al. (1996) has been clarified, on statistical grounds, by Kong et al. (1997). According to the latter, the NIDDM2 linkage result fails to "meet criteria for genome-wide significance for an anonymous locus" (Kong et al. (1997, p. 148). We also failed to replicate the suggestive linkages, reported by Hanson et al. (1997), between diabetes and regions on chromosomes 1 (1q21-23), 7 (7q21-22), and 11 (11q23-25). Neither the studies described above nor two other studies involving the Mexican American population (Mitchell et al. 1998; Sakul et al. 1998) have reported evidence for linkage of diabetes to the chromosome 10q region implicated in the present study. It is worth noting, however, that a recent genomewide scan for type 1 diabetes in a large Bedouin Arab family has provided evidence for a diabetes-susceptibility locus that maps to chromosome 10q25 (Verge et al. 1998), the region of interest in the present study.

We have previously reported evidence for linkage of 2-h plasma-glucose levels to a genetic location (D11S899/D11S1324) on chromosome 11p, near the β-cell high-affinity sulfonylurea receptor 1 (SUR1) locus (Stern et al. 1996*a*). SUR1 plays an important role in glucose-induced insulin secretion, and point mutations of the SUR1 gene have been found in subjects with familial persistent hyperinsulinemic hypoglycemia of infancy, a disorder of unregulated insulin secretion associated with severe neonatal hypoglycemia (Thomas et al. 1995; Nestorowicz et al. 1996). In the present analysis, the D11S899/D11S1324 region is weakly linked to both the age-at-onset-of-diabetes phenotype (LOD score 0.85) and the diabetes phenotype (LOD score 0.86). There is some evidence of association between two polymorphisms of the SUR1 gene and type 2 diabetes in whites (Inoue et al. 1996; Hani et al. 1997*a*). Several studies, however, have concluded that the SUR1 locus is not a major determinant of type 2 diabetes in populations such as whites (Linder et al. 1997), Japanese (Ohta et al. 1998), and Mexican Americans (Stirling et al. 1995).

In the chromosome 10q region where the strongest evidence for our linkage signal was found, there is no known candidate gene for susceptibility to type 2 diabetes. Neither do the genetic locations on chromosomes 3 and 9, where suggestive linkages were found, contain any known candidate genes for diabetes. In a recent genomewide scan for diabetes-susceptibility genes in another Mexican American population, a region on chromosome 3p (3p21.1-21.3) has been shown to contain a diabetes-susceptibility gene (LOD score 2.6) (Sakul et al. 1998). One of the chromosomal regions of interest in the present study is also on chromosome 3p; however, it is centromeric to the region 3p21.1-21.3. Elbein et al. (1997) have found significant evidence for linkage of fasting glucose level to a genetic location on chromosome 9p (LOD score 3.95), 13 cM from 9p-ter. This region was linked neither to type 2 diabetes nor to postchallenge glucose concentrations. However, it should be noted that the chromosome 9p genetic location of interest in the present study is ~15 cM from 9p-ter. The suggestive linkage of a location near the markers D4S1615 and D4S175 to type 2 diabetes may implicate intestinal fatty acid–binding protein, which has been shown to be both associated with insulin resistance in Pima Indians (Baier et al. 1995) and linked to 2-h insulin concentrations in Mexican American families (Mitchell et al. 1995).

Since genomewide scans for linkage involving complex disease phenotypes may pose the problem of false-positive results, several suggestions have been made by various researchers to verify claims of linkage. For example, Lander and Kruglyak (1995), in reference to the allele-sharing methods, proposed the term "significant linkage" to refer to LOD scores for various categories of relative pairs approximately in the range 3.3–3.8 and proposed the term "suggestive linkage" for LOD scores approximately in the range 1.9–2.4. According to Ter-

williger et al. (1997), "true" peaks appear to be relatively longer, and such "longer" peaks are more likely to harbor a susceptibility gene than are "shorter" peaks. The longer peaks may be indicative of the patterns of correlation between the adjacent marker loci shared IBD between relatives with linkage to a susceptibility locus. Our findings on chromosome 10q correspond to (*a*) a significant linkage between a genetic location near the marker D10S587 and age at onset of type 2 diabetes and (*b*) a suggestive linkage between the same chromosomal location and type 2 diabetes itself. Furthermore, both the diabetes analysis and the age-at-onset-of-diabetes analysis yielded similar linkage profiles, involving longer peaks.

Data simulations have demonstrated that extended pedigree–based multipoint variance-components approaches have greater power to detect linkages than do nuclear family–based approaches, especially for quantitative traits (Williams et al. 1997; Duggirala et al. 1997; Almasy et al. 1997). Such increased power may be partly due to the additional meioses available in extended pedigrees and to the increased ability to determine marker phase (Wijsman and Amos 1997). Aside from relative efficiency (Greenberg et al. 1996, 1997; Farrall 1997; Elston 1998), the extended pedigree–based multipoint variance-components approach differs from the commonly used affected-sib-pair methods, in the following ways. The variance-components approach uses information not only from the affected relative pairs but also from the unaffected and discordant relative pairs across generations in complex pedigrees. After the effects of age have been taken into account, as has been done in our discrete-trait analysis, the unaffected individuals provide valuable information for linkage; in particular, the older they are, the more linkage information they provide. With regard to age at onset, information from increasingly distant relative pairs (e.g., first cousins) may, relative to sib pairs, reduce the problem of possible cohort effects on onset times and may provide increased power to detect linkage. Although age-at-onset information obtained through self-report may be imprecise, the colocalization of the peaks for both the diabetes phenotype and the age-at-onset-of-diabetes phenotype is reassuring.

The lack of replicability of initial linkage claims by various research groups is of concern. If it can be assumed that these claims are not false positives, then the discrepant results across the studies imply substantial genetic heterogeneity underlying the phenotypic expression of type 2 diabetes in diverse ethnic groups. It is possible that the common form of type 2 diabetes is truly oligogenic, with loci having differential effect sizes, and that some forms of diabetes may indeed be caused by genes that are rare but that have appreciable effects. Also, replication of a true linkage in a disease with oligogenic inheritance is difficult because it is likely to require a larger number of families than is required for the initial detection (Suarez et al. 1994). This is because the fortuitous circumstances that led to detection of the linkage in the original set of families may not be replicated for oligogenic traits unless many more families are examined in the replication effort.

In conclusion, by conducting a genomewide scan for type 2 diabetes–susceptibility genes in a Mexican American population, we have found the strongest evidence for linkage, both to age at onset of diabetes and to type 2 diabetes itself, at a genetic location on chromosome 10q. This susceptibility locus appears to have substantial influence on the phenotypic variation of both type 2 diabetes and age at onset of type 2 diabetes. Since there are no candidate genes in this region, positional cloning efforts would be required to identify a diabetes-susceptibility gene. Confirmation of our results in other populations would strengthen the argument for undertaking such a positional cloning project.

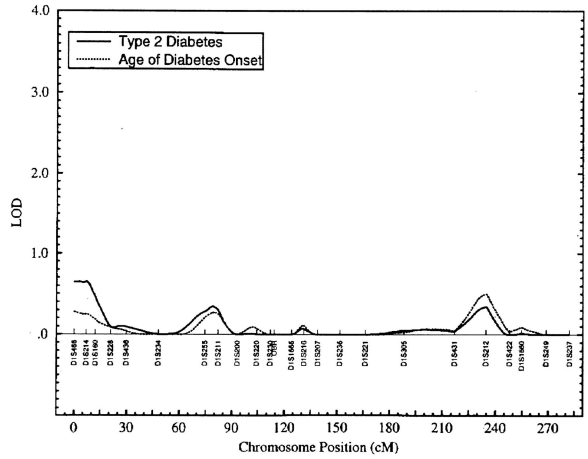
Acknowledgments

This research was supported by National Institutes of Health grants DK47482, DK42273, and DK44297. L.A. was supported by National Institutes of Health grant GM18897. We wish to thank Rajeswari Cheruvu, Edgardo Benavides, Stephanie Fleming, and Bonnie Reus for technical assistance. We wish also to thank Dr. Mary Pat Moyer and Ms. Florence Wall for establishing the lymphoblastoid cell lines. We also would like to thank Sharon Gaskill and Richard Granato for coordinating the field work. We warmly thank the SAFADS participants for their enthusiasm and cooperation.

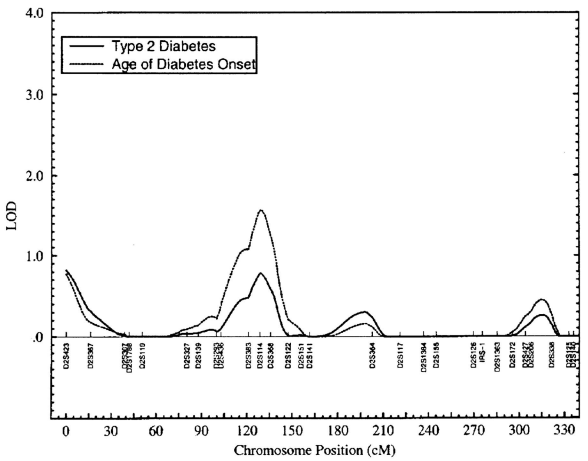
Appendix

Multipoint Linkage Results for Type 2 Diabetes and Age at Onset of Diabetes, by Chromosome

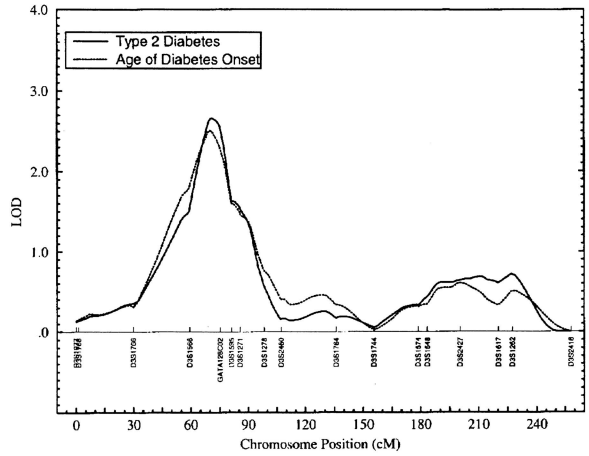
Chromosome 1



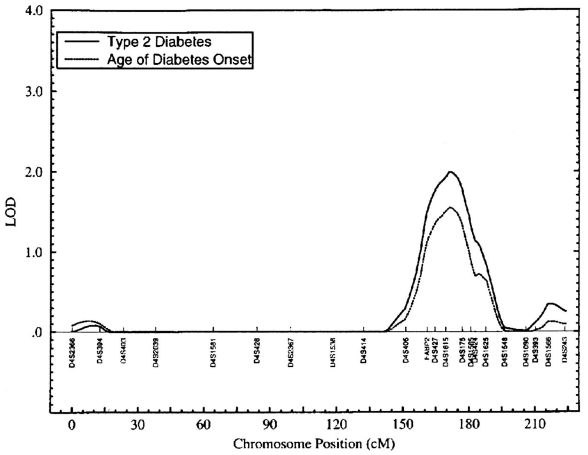
Chromosome 2



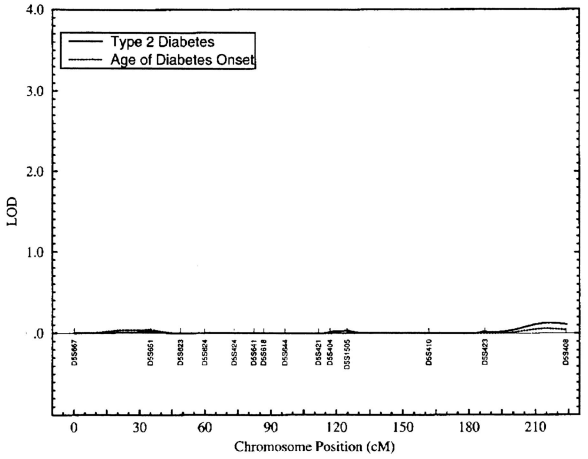
Chromosome 3



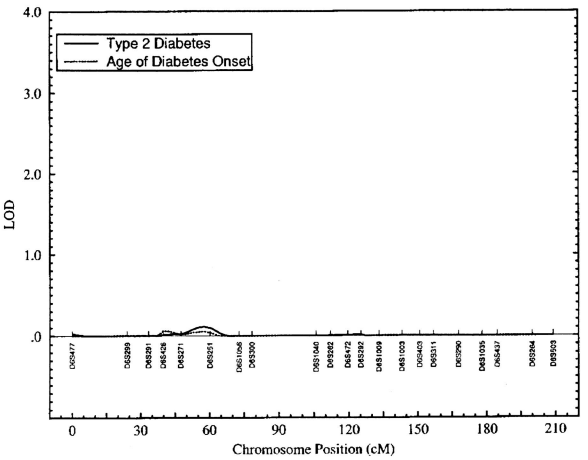
Chromosome 4



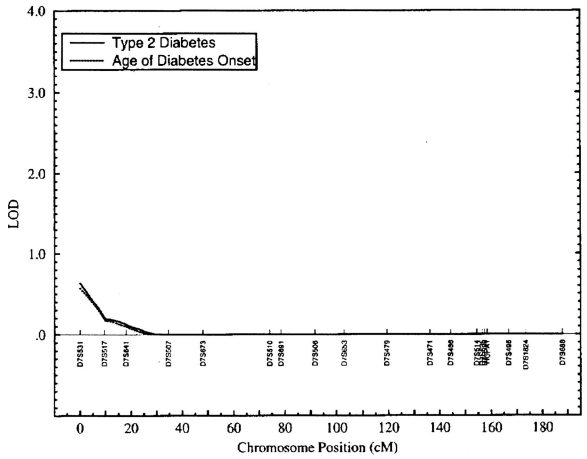
Chromosome 5



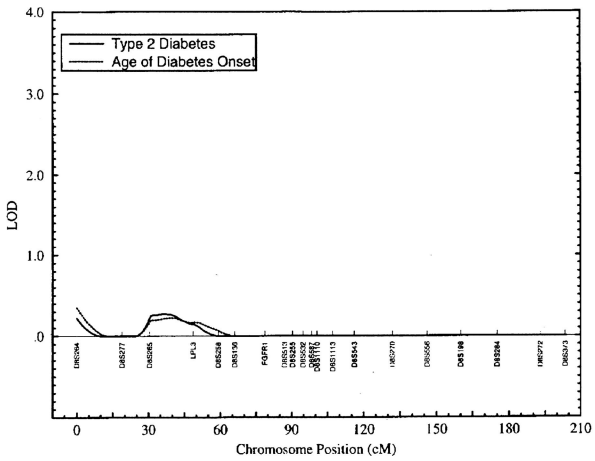
Chromosome 6



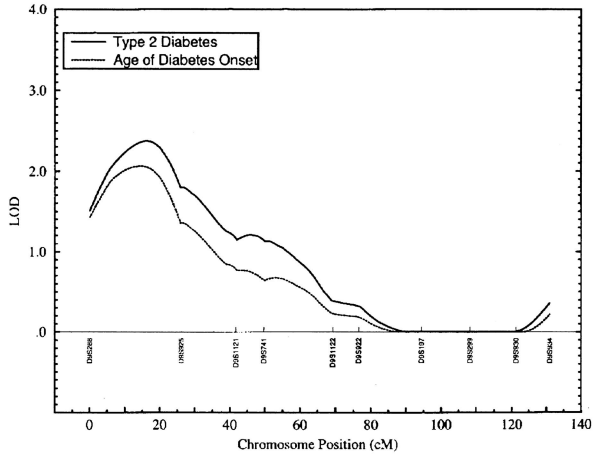
Chromosome 7



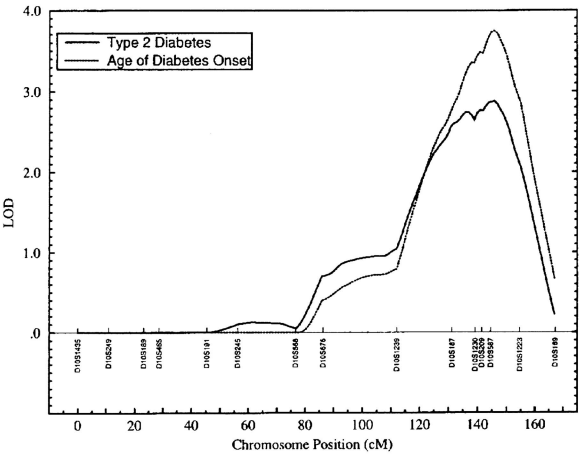
Chromosome 8



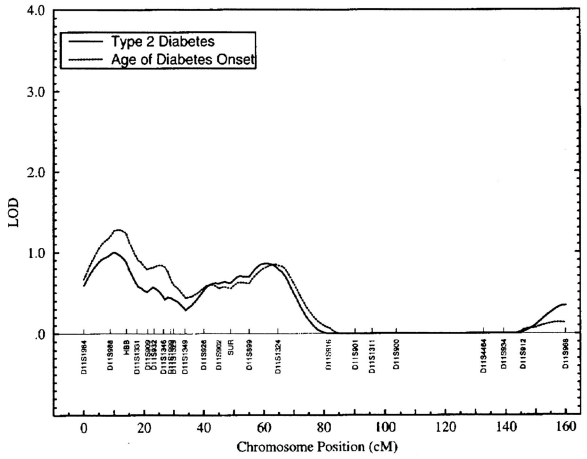
Chromosome 9



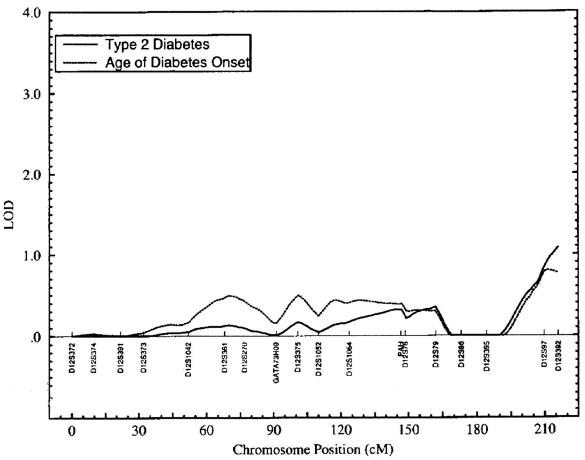
Chromosome 10



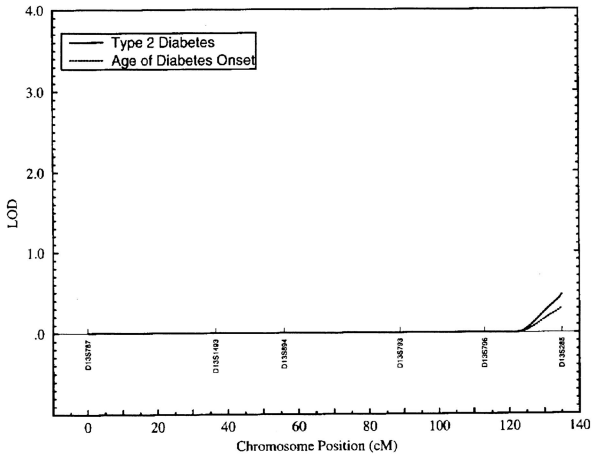
Chromosome 11



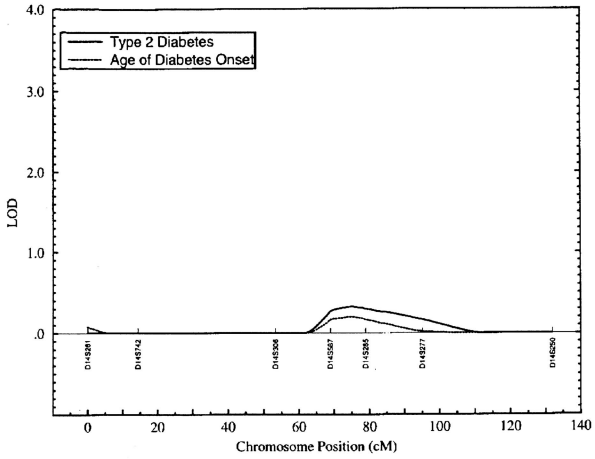
Chromosome 12



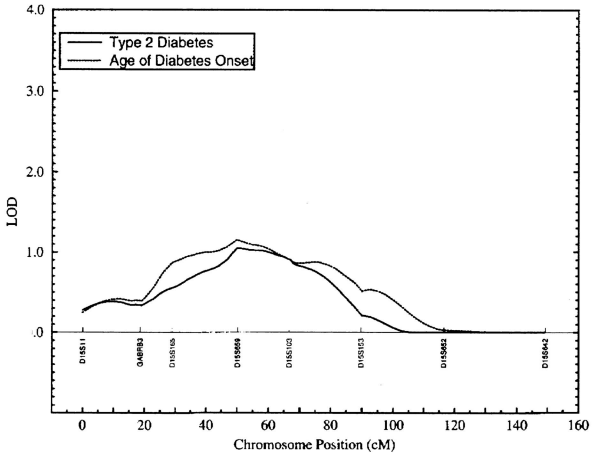
Chromosome 13



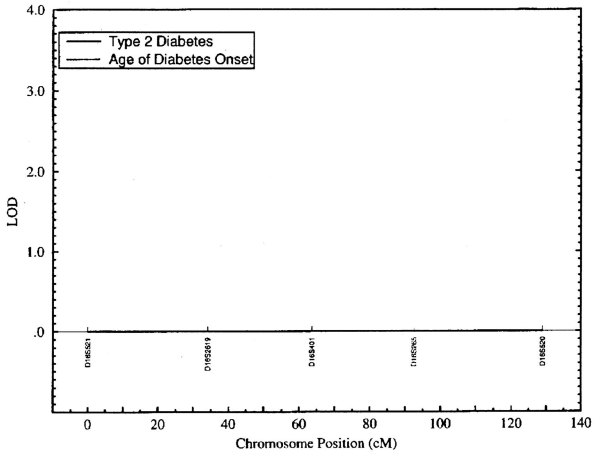
Chromosome 14



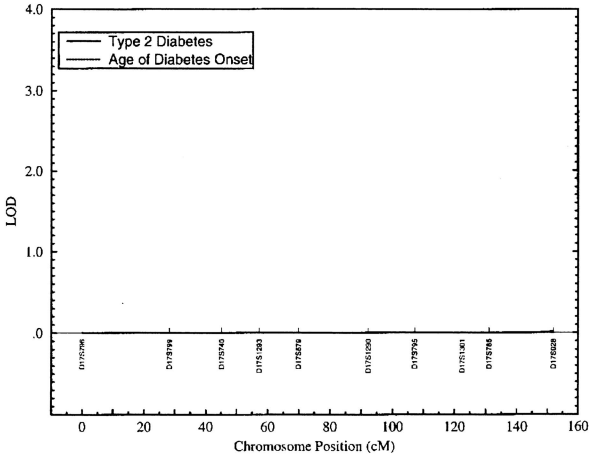
Chromosome 15



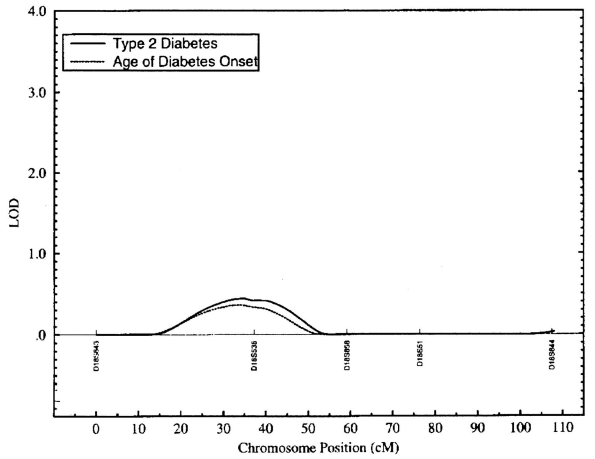
Chromosome 16

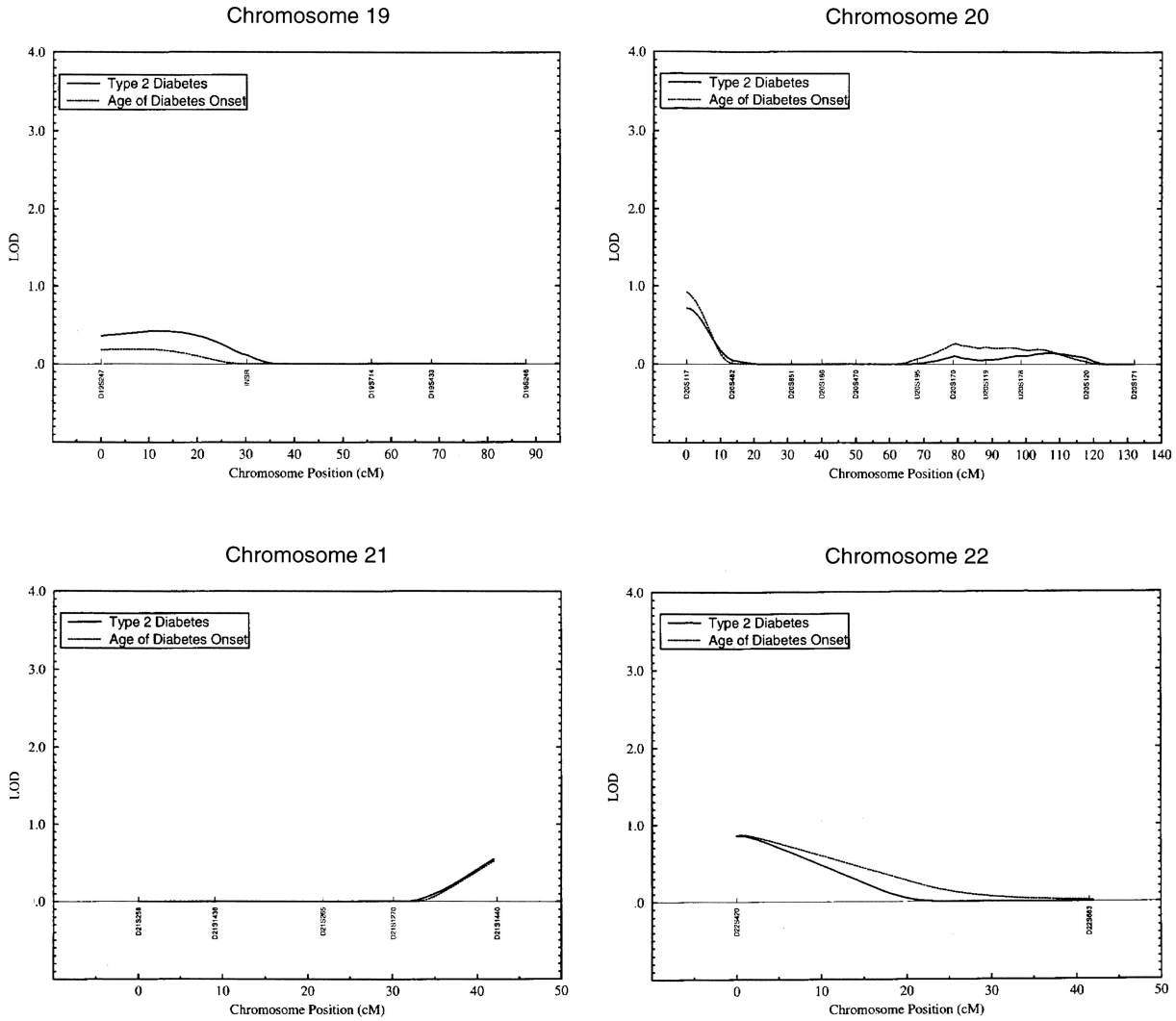


Chromosome 17



Chromosome 18





Electronic-Database Information

URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org
Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html/ summaryml.html
MARS, http://mars.uthscsa.edu/POClab/NIDDM/gensearch.html

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