Linkage Studies on NIDDM and the Insulin and Insulin-Receptor Genes

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Twenty Black families in which at least two siblings had non-insulin-dependent diabetes mellitus (NIDDM) were typed for restriction-fragment–length polymorphisms at the insulin (*INS*), insulin-receptor (*INSR*), and *HLA-DR*β loci. Evidence for linkage between NIDDM and these loci was assessed with various genetic models for the transmission of NIDDM and with the affected–sib–pair approach, which does not require assumptions concerning a genetic model for NIDDM. Tight linkage between NIDDM and any of the loci was unlikely under all of the genetic models examined. Similarly, for all three of the loci, the distribution of affected sib pairs sharing 2, 1, or 0 genes identical by descent was not significantly different from (and was very similar to) that expected if the locus were unrelated to disease susceptibility. There was no evidence for linkage heterogeneity for any of the loci when families were grouped according to obesity or age at onset or when considering families individually. We conclude that the *INS* and *INSR* loci can be ruled out as major susceptibility loci for NIDDM in most Black families segregating this disorder, but we recognize that defects at either of these loci may cause or contribute to NIDDM in some patients. In addition, it is possible that variation at the *INS* and/or *INSR* loci may contribute to NIDDM susceptibility by modifying susceptibility due primarily to another major gene(s) or as part of an overall polygenic component to NIDDM. *Diabetes* 38:653–58, 1989

Glucose 1 mM = 18 mg/dl	C-peptide 1 nM = 3.02 ng/ml

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Non-insulin-dependent diabetes mellitus (NIDDM) is a highly familial disorder. Twin and family studies have shown that there is a genetic component to NIDDM (1–3), and specific defects in genes involved in carbohydrate metabolism have been identified in some NIDDM patients (4,5). However, the genetic component of NIDDM susceptibility in most patients remains poorly characterized. NIDDM is almost certainly etiologically and genetically heterogeneous, with several genetic and environmental factors contributing to susceptibility to disease. Nevertheless, one or a few genes may account for much of the susceptibility to NIDDM in an appreciable subgroup of patients. The identification of a gene (or genes) that contributes to NIDDM susceptibility would have profound implications for the treatment and prevention of this major public health problem.

Genetic linkage studies are among the most effective ways to identify major susceptibility genes for complex disorders such as NIDDM and to delineate genetically homogeneous subgroups of patients and their families. The availability of DNA probes that detect restriction-fragment–length polymorphisms (RFLPs) near several NIDDM candidate genes makes this strategy feasible. We have collected data on 20 Black families in which NIDDM is segregating and report on results of linkage studies on two candidate genes for NIDDM, the insulin (*INS*) and insulin-receptor (*INSR*) genes. Although a linkage study of *INS* and NIDDM in a White sample has been previously reported (6), and a study on *INSR* in a pedigree ascertained through a former gestational diabetes patient was reported (7), there are no published linkage studies of *INS* or *INSR* and NIDDM in a sample of Black subjects.

In contrast with the well-characterized association between insulin-dependent diabetes mellitus (IDDM) and HLA-B and -D region antigens, there are no associations between HLA antigens and NIDDM in White (8) or U.S. Black (9) populations, and the HLA region is unlikely to play a major

role in NIDDM susceptibility. However, there are no published linkage studies of NIDDM and HLA. Consequently, we expected that the linkage analyses of NIDDM and the *HLA-DR*β locus from this study would provide the first direct confirmation and that this aspect of the study could serve as a negative control for comparisons with results from linkage analyses on *INS* and *INSR*. Such comparisons supplement the formal statistical tests of genetic heterogeneity within NIDDM with respect to *INS* and *INSR*.

Linkage studies of complex disorders such as NIDDM present methodological difficulties not encountered with fully penetrant mendelian diseases. Because the age at onset of NIDDM is usually late (after age 30 yr), the parents of affected subjects may no longer be living and their children may provide little information because they are too young to be affected. The mode of transmission of NIDDM is not known but must be specified by genetic parameters (e.g., allele frequencies and penetrances for the susceptibility locus) for the likelihood calculations usually used to assess the evidence for linkage. Results of linkage analyses will partly depend on values assumed for these parameters. Therefore, we used various approaches to assess the evidence for linkage of NIDDM with *INS*, *INSR*, and *HLA-DR*β. We performed the likelihood calculations for linkage analysis with several different assumptions for the inheritance of NIDDM. Previous studies have suggested that this strategy can be used to ensure that a true linkage is not missed in the analysis of a disorder with a complex and unknown mode of transmission (10–12). We also examined our data by the affected–sib-pair approach, which does not require assumptions about the mode of transmission.

MATERIALS AND METHODS

Families. Families ascertained for this linkage study were required to have at least two sibs with NIDDM and at least two additional sibs, affected or unaffected, available for study. Data were also collected on any living parents of the sibship members. The requirement for the two additional sibs was included to increase the ability to draw genetic inferences in sibships in which one or both parents were deceased. If any child of members in the sibship had NIDDM, data were collected on all available children of that member.

Subjects at the Rodebaugh Diabetes Center at the Hospital of the University of Pennsylvania were screened with a family history questionnaire, and those whose families met the ascertainment requirements were contacted for participation in the study. Most of the subjects in the clinic population are Black. Although there was no intent at the start of the study to restrict the data collection to Black families, all subjects whose families met criteria for the study, lived in the Philadelphia area, and were willing to participate were Black; the sample consisted of 20 Black families. This study was approved by the University of Pennsylvania Committee on Studies in Human Beings.

Information on all subjects was obtained by history and included height, weight, age, and medications currently taken. Percentage of ideal body weight was calculated from the Metropolitan Life Insurance tables (13). No subjects were pregnant or taking glucocorticoid medications at the time of the study. None reported a history of pancreatitis. Subjects who reported a diagnosis of diabetes stated their age at diagnosis and type of treatment.

Thirty milliliters of heparinized blood was obtained for DNA extraction, and 5 ml of blood, preserved with sodium fluoride and potassium oxalate, was obtained at least 2 h after eating for determination of glucose concentration. Plasma glucose was determined by a glucose oxidase method (Beckman Glucose Analyzer II, Fullerton, CA).

Determination of diabetes was made with a practical modification of the National Diabetes Data Group criteria (14). A plasma glucose concentration <140 mg/dl was required to substantiate a reported history of no diabetes. Subjects without a reported history of diabetes but with a glucose concentration ≥140 mg/dl after 6 h of fasting or ≥200 mg/dl 2 h postprandial were considered to have diabetes. A history of reported diabetes was confirmed by these same glucose concentration criteria or, alternatively, a similar report of blood glucose concentrations from physician records.

Of the 61 subjects diagnosed as having NIDDM for this study, 58 were receiving ongoing treatment for NIDDM; the 3 newly diagnosed diabetic subjects had plasma glucose concentrations (mg/dl) of 200 (fasting) and 215 and 250 (postprandial), and all 3 were symptomatic. Subjects classified as nondiabetic for this study had plasma glucose concentrations (mg/dl) ranging from 71 to 125 (postprandial) and from 68 to 122 (fasting).

No diabetic subject reported a history of ketoacidosis. A diligent effort was made to obtain the medical records of all insulin-treated diabetic subjects. Review of available records uncovered two patients with a history of ketoacidosis during an episode of serious febrile illness. C-peptide determinations in these patients, however, were ≥3.0 ng/ml, making a diagnosis of typical IDDM unlikely.

Although we are confident that all subjects considered affected for this study have NIDDM, we recognize that with more stringent testing some of the family members currently classified as unaffected might be found to be nonnormal or frankly diabetic. For both the parametric and affected–sib-pair linkage analyses, the consequences of misclassifying affected subjects as unaffected are far less serious than the consequences of misclassifying unaffected subjects as affected. Therefore, we believe our diagnostic criteria are both prudent and appropriate.

DNA studies. Two probes for the *INS* region were used. A probe specific for the hypervariable region (HVR) located 363 base pairs (bps) 5′ to the coding region of the *INS* was provided by G.I. Bell (Howard Hughes Medical Institute, Univ. of Chicago, Chicago, IL; 15). Although various enzymes can be used to detect the length variation in the HVR, *Pvu*II is the most informative because it cuts just 5′ and just 3′ to the HVR. An additional *INS*-region probe, provided by S. Elbein (Univ. of Utah, School of Medicine, Salt Lake City, UT; 16), detects high-frequency *Taq*I- and *Rsa*I-site polymorphisms located ~11 and 13 kilobase pairs (kbps) 5′ to the insulin gene. The cDNA probes for the *INSR* were provided by O. Rosen (Sloan Kettering Cancer Center, New York; 17). We used an ~1000-bp *Eco*RI fragment from the α-subunit region, which includes the putative ligand-binding domain, and a ~1600-bp *Pst*I fragment from the β-subunit region, which includes the putative tyrosine kinase domain. The α-probe detects high-frequency RFLPs with *Pst*I (two

RFLPs) and *Rsa*I, and the β-probe detects high-frequency RFLPs with *Bam*HI, *Bgl*II, *Kpn*I, and *Rsa*I. One of the *Pst*I RFLPs is described by Sanna et al. (18), the *Bam*HI and *Bgl*II RFLPs are described by Elbein et al. (19), and the other *Pst*I, the two *Rsa*I, and the *Kpn*I RFLPs are described by Cox et al. (20). An ~520-bp *Pst*I fragment of a cDNA probe for the *DR*β-chain (21) was used for the *HLA-DR* studies. *Taq*I digests hybridized with this probe revealed informative DNA variation among and within *HLA-DR* types (22).

DNA was extracted from whole blood and digested with restriction enzymes, according to manufacturer's instructions (Bethesda Research, Gaithersburg, MD). Digested DNA was electrophoresed in 1.0% (for detection of the *INS HVR* RFLP) or 0.7% (for all other RFLPs) agarose for 16–26 h at 40–70 V and blotted to 20 × 22-cm nylon membranes (Zetabind, AMF Cuno, Meriden, CT). Membranes were prehybridized overnight and then hybridized for 16–20 h at 65°C in fresh hybridization solution containing ^{32}P-labeled probe at a concentration of 2–5 × 10^6 cpm/ml. Membranes were washed twice for 1 h at 65°C with 0.1% saline sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) (or 0.5% SSC/0.1% SDS for *Rsa*I with the *INSR* α-probe) and placed in cassettes with one intensifying screen and XAR-5 film for 1–7 days at −70°C.

RFLPs at the *INS* and *INSR* loci were serially typed in each family until the family was fully informative for linkage studies [i.e., until all 4 parental chromosomes at the locus could be unambiguously identified or until the RFLPs at that locus were exhausted (23)]. Family members were always run on the same gels, and the large size of the gel (and membrane) insured maximum discrimination of bands for the highly polymorphic *INS* and *DR*β loci. The segregation of bands detected with the *INS* and *DR*β probes were also used to verify paternity.

Linkage analyses. Linkage analyses were performed with a version of the computer program LIPED (24) modified to allow for sex- and age-dependent penetrances (25). Because there are no other analyses of the segregation of NIDDM in Black families that would provide parameters for the mode of transmission of NIDDM, we assessed the evidence for linkage with various genetic models to ensure that a true linkage would not be missed. The models are summarized in Table 1. In all models, we assume that there is a locus with two alleles (*D1* and *D2*), one of which (*D2*) increases susceptibility to NIDDM. A genetic model is fully specified by the frequency of the susceptibility allele, q_{D2} (with $q_{D1} = 1 - q_{D2}$), and the penetrances for the three genotypes, *f*(*D1D1*), *f*(*D1D2*), and *f*(*D2D2*), where the penetrance for a genotype is defined as the probability of being affected given that genotype. For example, in a fully penetrant autosomal-dominant model, *f*(*D1D1*) = 0, *f*(*D1D2*) = 1, and *f*(*D2D2*) = 1, whereas in a fully penetrant autosomal-recessive model, *f*(*D1D1*) = 0, *f*(*D1D2*) = 0, and *f*(*D2D2*) = 1. The five penetrance sets summarized in Table 1 were chosen to cover a wide range of possibilities for the inheritance of NIDDM: two are recessivelike models (3 and 4) with equal penetrances for the nonsusceptible homozygote (*D1D1*) and heterozygote (*D1D2*), two are intermediate models (1 and 5) with the heterozygote (*D1D2*) penetrance between those for the homozygotes (*D1D1* and *D2D2*), and one is dominantlike (model 2) with equal penetrances for *D1D2* and *D2D2*. Several considerations determined the particular set of penetrances chosen for each model. Although the mode of transmission of NIDDM is not known, NIDDM is almost certainly etiologically heterogenous. To accommodate this heterogeneity, we allow the penetrance for the *D1D1* (nonsusceptible) homozygote to be nonzero in all five penetrance sets. That is, even individuals with no susceptibility alleles from the susceptibility locus have some probability of developing disease because of the contribution from other susceptibility loci for NIDDM (for example) or because of effects from environmental factors that increase susceptibility to NIDDM. Similarly, because the monozygotic-twin concordance for NIDDM is <1 (3), we allow for incomplete penetrance in the susceptible genotypes (*D1D2* and *D2D2*) for most of the models. Finally, for each genotype, the penetrance was set higher for females than for males to reflect the higher lifetime prevalence (K_p) of NIDDM reported in females (26). K_p can be calculated from the penetrances and allele frequencies as

$$K_p = q_{D1}^2[f(D1D1)] + 2(q_{D1}q_{D2})[f(D1D2)] + q_{D2}^2[f(D2D2)].$$

Because a range of values for K_p in Blacks has been reported (27), we tested each of the five penetrance sets with two susceptibility allele frequencies. One was calculated from the above equation by fixing the K_p to a reasonable lower limit, and the other was calculated from the above equation by setting K_p to a reasonable upper limit. A straight-line age-at-onset correction, with penetrances increasing linearly from 0 at age 13 yr to the maximum for the genotype at age 80 yr, was used in all linkage analyses. Previous studies have shown that the precise form of an age-at-onset correction (e.g., straight line, cumulative normal, or empirical) in general has little effect on results of analyses; what is important is to employ a reasonable correction, if the disease

TABLE 1
Assumptions of genetic models used in linkage analyses of NIDDM

	Model no.				
	1	2	3	4	5
f(*D1D1*)*					
Male	0.001	0.001	0.001	0.001	0.001
Female	0.002	0.002	0.002	0.002	0.002
f(*D1D2*)					
Male	0.400	0.500	0.001	0.001	0.070
Female	0.600	0.700	0.002	0.002	0.150
f(*D2D2*)					
Male	1.000	0.500	0.650	0.350	0.750
Female	1.000	0.700	0.850	0.500	0.900
Low q_{D2}	0.020	0.015	0.160	0.210	0.070
Male K_p	0.017	0.016	0.018	0.016	0.014
Female K_p	0.026	0.023	0.024	0.024	0.026
High q_{D2}	0.050	0.050	0.280	0.350	0.150
Male K_p	0.041	0.050	0.052	0.044	0.035
Female K_p	0.061	0.070	0.068	0.063	0.060

*We assume a disease susceptibility locus for NIDDM that has 2 alleles, *D1* and *D2*, with frequencies q_{D1} and q_{D2} ($q_{D1} = 1 - q_{D2}$) and penetrances *f*(*D1D1*), *f*(*D1D2*), and *f*(*D2D2*), which are the probabilities of being affected for genotypes *D1D1*, *D1D2*, and *D2D2*, respectively. K_p, lifetime population prevalence.

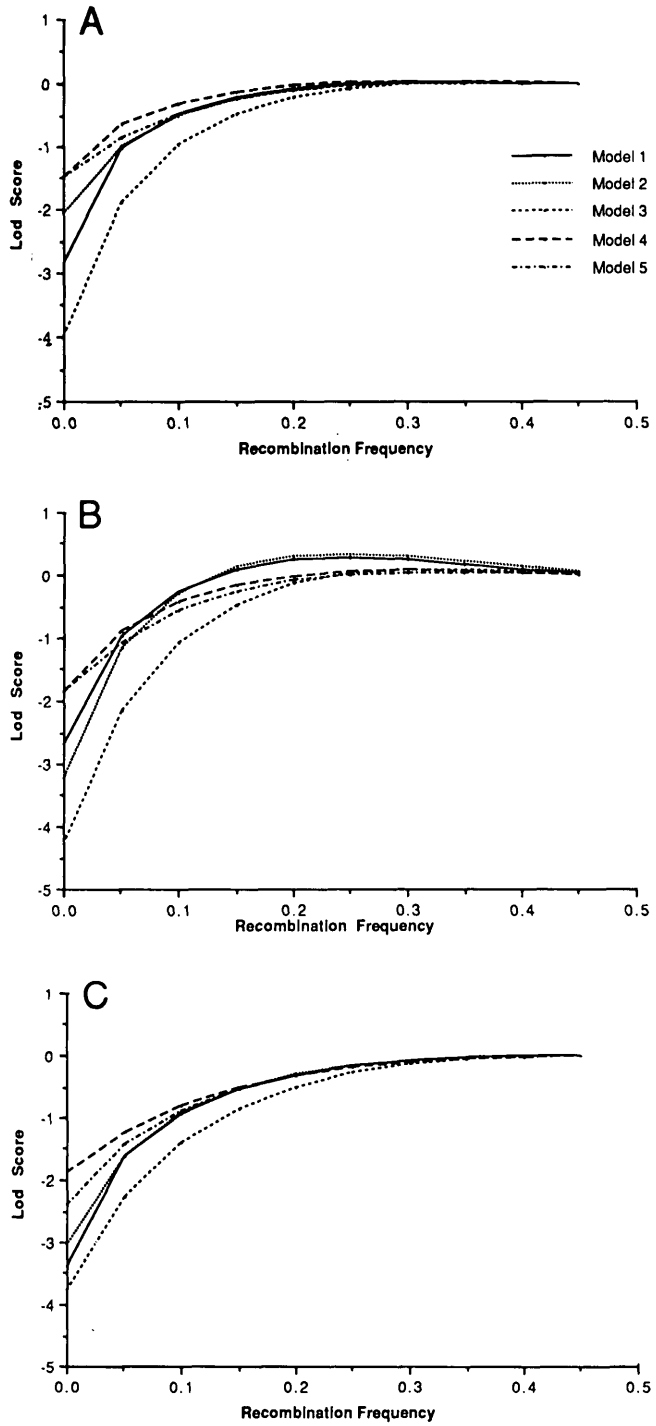

FIG. 1. Log of odds (lod) scores for NIDDM and the *HLA-DR*β (*A*), insulin (*B*), and insulin-receptor (*C*) loci for 5 models described in Table 1 with higher susceptibility allele frequency. Lod scores for each model were summed for all families.

demonstrates a significantly increased risk of expression with age (28).

Evidence for linkage heterogeneity was assessed with the k-sample heterogeneity test (29). We tested for subsets of families defined by obesity (families with no nonobese diabetic members vs. families with at least one nonobese diabetic member) and age at onset (families with no diabetic members with onset before age 35 yr vs. families with at least one diabetic member with onset before age 35 yr).

We also tabulated the proportion of genes identical by descent (IBD) in affected sib pairs. If susceptibility to disease is unlinked to a marker, the proportion of affected sib pairs who have two, one, or zero genes IBD at that marker is expected to be 1/4, 1/2, and 1/4, respectively. If the marker is linked to a locus that increases susceptibility to disease, however, the proportion of affected sib pairs who share marker genes IBD is increased. The precise deviation from the null distribution depends on the recombination frequency between the marker and the disease-susceptibility locus and the inheritance of disease susceptibility (i.e., penetrance and allele frequency). The affected–sib-pair approach can be used to assess the evidence for linkage between a disease and a marker simply by comparing the observed distribution of shared genes in affected sib pairs to that expected in the absence of linkage. The advantage of this approach over the linkage-likelihood analysis is that it does not require assumptions about the mode of transmission of disease susceptibility; the disadvantages of this approach are that it does not yield an estimate of the recombination frequency and it has less power than likelihood analyses, at least in some areas of the genetic parameter space.

RESULTS

Description of family data. Data were collected on 101 subjects in 20 families. Although all 20 families met the initial criteria for study, some family members moved, died, or changed their minds about cooperating and could not be studied. In addition, 4 subjects from three sibships were excluded for nonpaternity. All 4 of these subjects demonstrated inconsistencies with the *DR*β and *INS* loci, and 3 of the 4 had inconsistencies detectable in haplotypes constructed from the slightly less informative *INSR* locus. The remaining 97 subjects were included in the analyses. Four or more sibs were studied in 15 of the families, 3 sibs were studied in 4 of the families, and only 2 sibs were studied in 1 family. However, additional family members (parents or children of sibship members) were studied in 8 families, including 2 of the 4 families in which only 3 sibs were studied.

A slightly higher proportion of females (38 of 58, 66%) than males (23 of 39, 59%) had been diagnosed with NIDDM in these families, in agreement with studies on unrelated subjects, suggesting that females are at higher risk of developing NIDDM. Affected individuals did not differ significantly from their unaffected family members in percentage of ideal body weight for height, although more affected than unaffected family members were clinically obese (>20% over ideal body weight for height) at the time of study. The average age at onset of diabetes in affected family members was 43.8 yr and ranged from 13 to 73 yr. (The 13-yr old was the only diabetic subject with onset before age 20 yr and had no evidence for a diagnosis of IDDM.) Most family members with onset of NIDDM before age 35 yr were female (15 of 18), and these females were significantly more obese at the time of study than those with onset after age 35 yr.

All of the DNA band patterns segregating in the families had been previously observed in control populations; we therefore concluded that there are no gross deletions or rearrangements of DNA in the *INS*, *INSR*, or *HLA-DR* regions in these families that might account for the transmission of

TABLE 2
Insulin, insulin-receptor, and *HLA-DR* gene sharing in all possible pairs of affected (NIDDM) sibs

	Fully informative: genes identical by descent			Partially informative: genes identical by descent	
Locus	2	1	0	At least 1 (2 or 1)	At most 1 (1 or 0)
Insulin	9	19	10	5	3
Insulin receptor	6	13	6	7	11
*HLA-DR*β	7	15	9	3	2

Families fully informative for determining identity by descent had 4 uniquely identifiable parental genes; in these families, sharing in affected sib pairs was unambiguous. Some families were not fully informative because <4 parental genes were identified or because there was >1 possible configuration for parental genes. In partially informative families, it was often possible to determine sharing for the contribution from 1 parent. Affected sibs from these families were classified as sharing at least 1 (i.e., 2 or 1) or at most 1 (i.e., 1 or 0) parental gene identical by descent. One family (3 affected sib pairs) was not informative for the insulin-receptor gene, and 3 families (10 affected sib pairs) were not informative or had affected members who were untyped for the *HLA-DR*β locus.

NIDDM (complete DNA data, pedigree structures, and clinical information for all subjects will be provided on request).

Results of linkage analyses were qualitatively similar, whether the lower or the higher disease prevalence models were used, but only the results for the high-prevalence models are presented. Figure 1*A* shows the log of odds (lod) score as a function of recombination fraction between NIDDM and *HLA-DR* for the five high-prevalence genetic models, with lod scores summed for the 20 families. Lod scores for all recombination frequencies were negative, and tight linkage between NIDDM and the *HLA-DR* region can be rejected for three of the five high-prevalence (and all 5 low-prevalence) models. Figure 1*B* and *C* summarizes results of the same analyses for the *INS* and *INSR* loci, respectively. Tight linkage to the *INS* locus can be rejected for 3 of the 5 high-prevalence (and all 5 low-prevalence) models; tight linkage to the *INSR* locus can be rejected for 4 of the 5 high-prevalence (and all 5 low-prevalence) models. In addition, the lod scores at a recombination frequency of 0 for the high-prevalence models, which did not allow conclusive exclusion of linkage (i.e., lod score greater than −2.0), were nonetheless quite low (less than −1.5), making tight linkage unlikely. Previous studies have found that when a marker is truly linked to a disease-susceptibility locus there is often evidence for linkage, even with genetic models that do not correctly specify the mode of transmission of disease (12). That linkage is so uniformly unlikely for all of these quite different genetic models indicates that the negative results are not likely to be due to a misspecification of the mode of transmission. As might be expected, the models providing the strongest evidence against linkage for all three loci are those with the highest penetrances (1, 2, and 3).

Tight linkage is similarly ruled out by the *INS* and *INSR* (and *HLA-DR*β) sharing in affected sib pairs (Table 2). The information obtained at these loci for all affected sib pairs is also summarized in Table 2. There was no evidence for linkage heterogeneity with the *INS*, *INSR*, or *HLA-DR* loci in the families subdivided by obesity or age at onset.

Genetic heterogeneity for NIDDM need not be reflected in these clinical features, of course. However, post hoc studies (i.e., looking for clinical characteristics that differentiate the families with positive lod scores from the families with negative lod scores) are difficult to interpret and require replication in an independent sample. An alternative is to use tests of heterogeneity that assume that each family is a separate subset, but these tests have little power. Lod score is presented as a function of recombination frequency between NIDDM and the *INSR* locus for one of the higher prevalence models (model 1), with the lod score curve of each family plotted separately (Fig. 2). If there is linkage heterogeneity for NIDDM at the *INSR* locus, with variation at this locus accounting for most of the NIDDM susceptibility in some appreciable subset of families, we would expect some families to show evidence for linkage, whereas the remainder of the families should show no evidence for linkage. However, even when a disease is not linked to a marker in any family, some families will have positive (though generally low) lod score curves by chance alone. The distribution of lod score curves for each family plotted separately with model 1 (high prevalence) for the *DR*β and *INS* loci was virtually identical to that shown in Fig. 2 for the *INSR* locus. If we take as established that NIDDM is not linked to *HLA*, the variation in lod score curves observed for the *DR*β locus must be similar to that expected by chance for a sample of this size (and with these same pedigree structures) when the disease is unlinked to the marker in any of the families. Because the distribution of family lod score curves in analyses of the *INS* and *INSR* loci are virtually identical to that found with the *DR*β locus, there appears to be no significant excess of families showing strong evidence for linkage between NIDDM and the *INS* or *INSR* loci. Results for all other models at both allele frequencies were qualitatively similar.

DISCUSSION

With various approaches, we found no evidence for linkage between NIDDM and the *INS* or *INSR* loci. Although results of this study provide no support for *INS* or *INSR* (or closely linked genes) as susceptibility loci for NIDDM, our ability to completely rule out these genes in NIDDM susceptibility is limited because of the complex nature of this disease. Be-

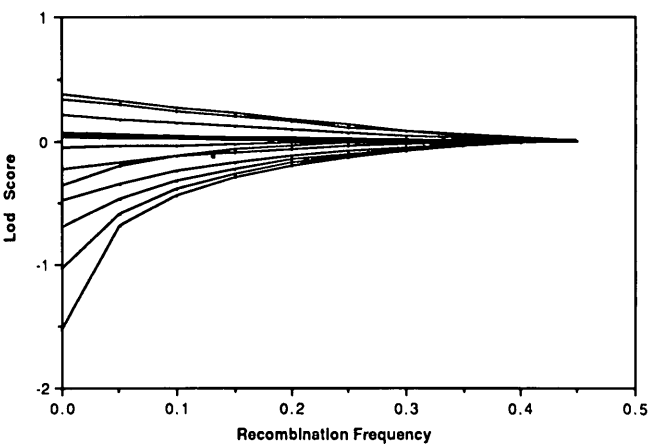

FIG. 2. Log of odds (lod) scores for NIDDM and insulin-receptor gene with model 1, higher frequency of susceptibility allele (see Table 1). The lod score curve for each family is shown.

cause there are gene mutations at the *INS* (4,30,31) and *INSR* (5,32) loci that cause or significantly increase the risk of NIDDM, it is clear that these genes can be major susceptibility factors in some individuals. In addition, linkage studies of complex diseases have modest power to detect linkages with loci of small effect; the number of families in our sample (or even much larger numbers of families) would not allow us to rule out the possibility that variation among the *INS* or *INSR* plays a minor role in NIDDM susceptibility by modifying susceptibility due mostly to some other locus or as part of a general polygenic component to NIDDM.

The RFLPs used as markers for these linkage studies detect DNA variation primarily in noncoding regions (introns and flanking regions) rather than in the coding regions of *INS* (15,16) or *INSR* (G.I. Bell and R. Taub, personal communication). However, recombination between the coding regions and the RFLPs should be very rare. Therefore, we would expect the estimated recombination frequency between NIDDM and the RFLPs to be 0 if the gene actually plays a major role in NIDDM susceptibility. Because tight linkage between NIDDM and the *INS* or *INSR* loci is very unlikely for all of the genetic models examined, our results allow us to rule out *INS* and *INSR* as major susceptibility loci for the majority of NIDDM in U.S. Blacks. Similar negative findings have been previously reported in linkage analyses of NIDDM with *INS* in a White sample (6). In addition, the results of heterogeneity tests on these data partitioned by obesity or age at onset and the comparison of our results on the *HLA-DR* region with results from the *INS* and *INSR* loci suggest that there is little evidence in our families for appreciable heterogeneity. Thus, if there are major susceptibility genes for NIDDM in Blacks, finding them will require studies on additional candidate genes and, perhaps, a systematic search with highly informative random probes.

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