New Susceptibility Locus for NIDDM Is Localized to Human Chromosome 20q

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To test the hypothesis that a gene (or genes) in the "MODY1 region" of the long arm of chromosome 20 contributes to the development of NIDDM, we conducted linkage studies in 29 extended Caucasian families in which many members were affected with NIDDM. A total of 498 individuals, including 159 NIDDM patients with an average age at diagnosis of 47 years, were genotyped for eight highly polymorphic microsatellite markers spanning a 31-cM region on chromosome 20q12-13.1. Using affected sib-pair analysis, we obtained evidence suggesting linkage between NIDDM and markers D20S119, D20S178, and D20S197 (allele sharing identical-by-descent [IBD], 0.56 for all three; $P = 0.005$, $P = 0.009$, and $P = 0.004$, respectively). Multipoint nonparametric linkage (NPL) analysis also showed evidence for linkage of NIDDM with the same three markers. The evidence for linkage was much stronger (allele sharing IBD by affected sibpairs, 0.64 [$P < 0.0001$]; maximum NPL score, 3.3 [$P = 0.009$]) in the 14 families whose average age at diagnosis of NIDDM was above the median (47 years) for all families. In these 14 families, one particular allele of the microsatellite D20S197 was transmitted from heterozygous parents to NIDDM offspring more frequently than expected ($P < 0.01$). This indicates that the marker allele and the disease allele are in linkage disequilibrium, implying that they are in close proximity. Consequently, the recently identified MODY1 gene (hepatocyte nuclear factor 4) is an unlikely candidate gene for NIDDM in our families, since it is located about 8 cM centromeric of D20S197. In conclusion, we have identified a new region on chromosome 20q that contains one or more NIDDM genes distinct from the recently identified MODY1 gene. *Diabetes* 46:876–881, 1997

Genetic susceptibility plays an important role in the development of NIDDM (1–3). To date, mutations in three genes have been found to be responsible for the development of maturity-onset diabetes of the young (MODY), a form of NIDDM that develops before 30 years of age and is inherited as an autosomal dominant disorder (4–8). While mutations in the glucokinase gene (MODY2) seem to be responsible for a mild form of MODY (4), more severe forms appear to result from mutations in the hepatocyte nuclear factor (HNF)-4 gene localized to the long arm of chromosome 20 (MODY1) (5,6) and in the HNF-1 gene localized to the long arm of chromosome 12 (MODY3) (7,8).

Efforts to identify susceptibility loci for the more common form of NIDDM occurring in middle and old age have been less successful (2,3,9–13). Although the linkage of NIDDM with two chromosomal regions has been found (14,15), these findings have not been reproduced (12,16). This lack of progress can be attributed to two factors. First, only a relatively small sample of NIDDM patients and families have been examined. Second, the identification of novel susceptibility genes or chromosomal regions is made difficult by the complex multigenic mechanisms of NIDDM inheritance (1).

To optimize the likelihood of finding NIDDM susceptibility loci, we have chosen to study extended families in which NIDDM has been diagnosed at between 30 and 60 years of age and segregates in a pattern consistent with an autosomal dominant disorder. This mode of inheritance has been supported by a recent segregation analysis (17) and has been found to represent the most common familial pattern in our survey conducted in patients of the Joslin Diabetes Center. Here, we report our results regarding the linkage between a candidate chromosomal region, the "MODY1 region" on chromosome 20q, and NIDDM in our panel of families.

RESEARCH DESIGN AND METHODS

Ascertainment and examination of NIDDM families. There are 17,700 active patients of the Joslin Diabetes Center who reside in Massachusetts. Among these are 7,500 patients whose diabetes was diagnosed at between 30 and 60 years of age. In 1993, questionnaires regarding family history of diabetes were sent to a 33% random sample. We obtained responses from 1,432 patients. In this group, there were 743 patients (probands) who had NIDDM treated at least for 2 years with diet or oral agents.

Based on information from the survey questionnaire, the group of NIDDM probands was divided according to family history of diabetes. Almost one half of the probands had one parent with diabetes, and 8% had two parents with diabetes. Within the group of probands having only one parent with diabetes, we identified 128 families in which the proband had at least one sibling with NIDDM. This group of families was evaluated further with regard to their suitability for genetic studies. In 55 families, diabetes was reported to occur in at

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ADA, adenosine deaminase; EBV, Epstein-Barr virus; HNF, hepatocyte nuclear factor; IBD, identical by descent; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; NPL, nonparametric linkage; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; TDT, transmission disequilibrium test.

least two generations with large numbers of family members available for study. A total of 29 families (all Caucasian) were examined out of those originally identified. The protocol for this study was approved by the Human Subjects Committee of the Joslin Diabetes Center.

Examinations were performed by trained family recruiters, and the majority of examinations took place in the home of the study participant. In addition to a standardized interview (including age at diagnosis of diabetes and history of its treatment) and the measurement of height, weight, and blood pressure, the participants were requested to have an oral glucose tolerance test (OGTT) to detect undiagnosed NIDDM. When an OGTT was not possible or patients had previously diagnosed diabetes that was treated with insulin or oral agents, a random glucose was obtained. Diagnoses of diabetes or impaired glucose tolerance (IGT) were based on World Health Organization criteria (18). For deceased parents and nonparticipating relatives, information about diabetes (age at onset and type of treatment) was obtained from informants (principally spouses and children) and, when possible, was verified by medical record review. For all study participants, DNA was isolated from peripheral lymphocytes or Epstein-Barr virus (EBV)-transformed lymphoblasts using standard protocols (19).

Genetic markers and genotyping. In 1991, Bell et al. (5) reported linkage between the adenosine deaminase (ADA) locus on chromosome 20 and diabetes in one large family (RW) with MODY. The locus segregating with diabetes in this family was designated MODY1. Subsequent fine mapping placed the MODY1 locus on chromosome 20q12-q13.1 to a 13-cM (sex-averaged) region located telomeric to the ADA locus (20–22) (Fig. 1). A recent study added markers centromeric to the ADA locus to the critical region (23), and while this manuscript was in review, the gene for HNF-4 located centromeric to ADA was determined to be responsible for MODY1 (6). Based on the Cooperative Human Linkage Center database (University of Iowa, Iowa City, IA) and the physical map of the MODY1 region, we selected eight markers for this study that span a 31-cM chromosomal region, including the expanded MODY1 region (23) (Fig. 1).

Genotyping for all markers was performed by polymerase chain reaction (PCR)-based protocols (25). Generally, the forward PCR primer was end-labeled with ($^{32}\gamma$)-P-ATP by T4 polynucleotide kinase (Promega, Madison, WI), according to the manufacturer's protocol. PCR was performed with a 10-µl volume in a 96-well PCR plate (MJ Research, Watertown, MA). Each PCR reaction contained 50 ng of genomic DNA, 10 pmol of each primer, 25 µmol/l deoxyribonucleoside triphosphates (dNTPs), 0.3–0.5 mmol/l $MgCl_2$, and 0.2 U of *Taq* polymerase (Roche Molecular Systems, Branchburg, NJ) with 1 µl of 10× PCR buffer. All PCR reactions were performed following the "hot-start" procedure by adding *Taq* polymerase and labeled primer mixture (in a total volume of 2 µl) after a denaturation step of 94°C for 5 min. Samples were subjected to 30 cycles of 40 s at 94°C for denaturing, 1 min at optimum annealing temperature, and 1 min at 72°C for elongation, using a 96-well Thermocycler (MJ Research). PCR reactions ended with 5 min of final incubation at 72°C. After PCR amplification, 2 volumes of sequencing stop solution was added to the PCR reaction, the mixture was heated at 95°C for 5 min, and 2 µl of the denatured PCR product was loaded onto 5% polyacrylamide DNA sequencing gels and resolved electrophoretically in 0.6× Tris-borate/EDTA (TBE) at 100-W constant current. Consistency among different gels and different lanes of the same gel was maintained by using either standard PCR products of known genotype or size standards from Research Genetics (Huntsville, AL). Dried gels were exposed to X-ray films for 12 h without using the intensifying screen. Autoradiograms were scored by two independent observers. PCR primers were purchased from Research Genetics (Huntsville, AL). Primer sequences were available from the Genome database (Johns Hopkins University). Allele frequencies of markers were estimated from 80 unrelated nondiabetic individuals (the majority being spouses of NIDDM patients).

Analysis of data. The program GENEHUNTER (26) was used to infer haplotypes for any parent who was deceased or otherwise unavailable for studies, but whose children (and frequently the spouse as well) were genotyped. Multiple analytical approaches were used to analyze the genetic data. These included nonparametric methods such as affected sib-pair analysis, multipoint nonparametric linkage analysis, and the transmission disequilibrium test.

Affected sib-pair analysis was performed, as the mode of inheritance of NIDDM cannot be specified with confidence. In this method, the average proportion of marker alleles identical-by-descent (IBD) that are shared by affected sibpairs was compared with 0.5, the expected proportion in the absence of linkage. If the marker is linked to the disease phenotype, a proportion >0.5 would be expected (assessed by a one-tailed significance test using a normal approximation to the binomial distribution). The analysis was performed two ways, one incorporating all possible affected sibpairs in sibships of size s, that is $s(s - 1)/2$, and the other restricted to the $(s - 1)$ independent affected sibpairs within each sibship (27).

Nonparametric linkage analysis was performed using the GENEHUNTER program (26), which performs multipoint analysis among all affected family members at each location along chromosome 20 that is spanned by D20S107 and D20S100. This approach calculates the nonparametric linkage (NPL) score at each position, comparing the observed IBD sharing among all affected family members with that expected under the null hypothesis of no linkage.

Evidence for association between NIDDM and alleles at the three most informative and strongly linked markers (D20S119, D20S178, and D20S197) was determined with the transmission disequilibrium test (TDT), as implemented in the GAS package, version 2.0 (Alan Young, Oxford University, 1993–1995). The TDT determines the frequency that an allele is transmitted from a heterozygous parent to an affected offspring (28). Unlike strict linkage tests, TDT only detects linkage in the presence of allelic association.

All analyses reported here were performed with affection status defined only for NIDDM. Individuals having IGT were considered as having an "unknown" phenotype.

RESULTS

We examined 29 NIDDM pedigrees that were ascertained through the following three characteristics: a proband with NIDDM diagnosed between 30 and 59 years of age, NIDDM present in at least one sibling of the proband, and one parent having diabetes according to the information available to the proband. The 498 individuals examined ranged from 15 to 95 years of age. Table 1 shows the distribution of examined individuals in families ranked according to the mean age at diagnosis of NIDDM. On average, five NIDDM individuals in one or two generations were examined in each family (range from 2 to 10 individuals). In addition, an average of 12 nondiabetic individuals (usually in two or three generations) were examined in each family (range 3 to 28 individuals). The mean age of the nondiabetic individuals at the time of examination was similar to the age at onset of NIDDM in their relatives. The nondiabetic individuals were selected because they were siblings of NIDDM individuals or were children or grandchildren of deceased NIDDM individuals. On examining the families more carefully, we found four of them to be bilineal, whereas in the others diabetes occurred on only one side of the family in at least two generations.

Comparisons of examined individuals according to diabetes status are shown in Table 2. Diabetes had been diagnosed in 159 subjects, 22 individuals had impaired glucose tolerance, and 317 were nondiabetic subjects. On average, the patients with NIDDM had diabetes diagnosed 13 years prior to this examination. At the time of examination, 39% of them were treated with insulin, 30% were treated with oral agents, and the remaining 31% were treated with diet or were untreated because diabetes had not been detected until this examination (n = 21). The subjects with diabetes had been very obese in the past (their lifetime maximum body weight averaged >150% of ideal) and were also more obese at the time of examination than their nondiabetic relatives.

After genotyping 498 individuals for eight highly polymorphic microsatellites, the genotypes of an additional 69 nonparticipating individuals (the majority of them dead) were inferred, including 39 diabetic subjects. Within the 29 pedigrees, 51 nuclear families with known parental haplotypes were identified. The distribution of these families according to the number of affected siblings was: 22 families with two, 11 families with three, 8 families with four, 5 families with five, 2 families with six, and 3 families with seven. A total of 246 affected sibpairs was formed within these families. Based on inferred as well as measured genotypes, the number of infor-

TABLE 1
Characteristics of the NIDDM families enrolled in the study according to mean age at diagnosis of NIDDM of the examined cases

	Individuals with NIDDM		Individuals without diabetes	
Pedigree identification	*n* (number of generations)	Mean age at diagnosis	*n*	Mean age at examination
09	3 (1)	36	10	42
22	2 (1)	36	4	63
04	4 (1)	39	13	31
33	2 (1)	40	7	42
05	7 (2)	42	20	47
49	9 (3)	43	4	44
10	8 (2)	43	5	44
40	4 (2)	44	3	39
45	7 (1)	45	3	45
31	2 (1)	46	5	48
11	10 (3)	46	19	34
37	7 (2)	46	28	48
36	6 (2)	47	7	52
21	7 (2)	47	16	51
17	6 (1)	47	19	44
47	4 (1)	48	6	39
06	5 (1)	48	26	51
48	4 (2)	49	13	38
23	4 (2)	49	10	54
26	7 (1)	50	10	54
32	2 (1)	50	7	50
44	10 (2)	50	18	47
07	5 (2)	52	13	59
46	5 (1)	52	17	49
43	6 (1)	53	9	54
18	8 (3)	53	14	50
19	5 (1)	55	19	46
27	6 (2)	56	9	53
01	4 (2)	63	5	65

Data are *n* or means.

mative pairs available ranged from 176 to 223, depending on the genetic marker. The proportion of alleles shared IBD by affected sibpairs for each marker is shown in Table 3. The mean proportion of alleles shared IBD was >0.50 for every marker studied, but the excess sharing was large only for D20S119, D20S178, and D20S197 ($P < 0.01$ for each), all telomeric to the ADA locus. The evidence for linkage became stronger when the families were stratified into two groups according to mean age at diagnosis of NIDDM within the family. There were 15 families with mean age at diagnosis of <48 years, and 14 families with mean age at diagnosis ranging from 48 to 63 years (Table 1). In this subgroup, the proportion of alleles shared IBD for the three central markers (D20S119, D20S178, and D20S197) ranged from 0.636 to 0.650, and the *P* values decreased to $<10^{-4}$. By contrast, in the younger families, the proportions of alleles shared IBD for these markers were 0.489, 0.478, and 0.480, respectively. The proportion shared did not deviate significantly from 0.5 for any markers in this subgroup.

Since our study included families with a large number of affected siblings, the results presented in Table 3 should be interpreted with caution because of the lack of independence among the sibpairs. To determine the proportion of alleles shared IBD after eliminating the correlation among the affected siblings, we analyzed only $(s - 1)$ independent affected sibpairs among the s siblings in each nuclear family. Allele sharing IBD at these three central markers was determined for 54 out of 59 independent sibpairs among the young-onset families and 54 out of 57 older-onset families. In this greatly reduced set of sibpairs, the pattern of allele sharing was unchanged. Among the older-onset families, allele sharing for these three markers rose slightly to 0.667, 0.676, and 0.660 (*P* values ranging from 0.0005 to 0.0001), while allele sharing ranged from 0.495 to 0.505 in the young-onset families.

TABLE 2
Clinical characteristics of the examined individuals according to NIDDM status

Clinical characteristic	Nondiabetic	IGT	NIDDM
n	317	22	159
Male (%)	47	23	54
Current age (years)	47 ± 18	59 ± 16	60 ± 12
Age at diagnosis (years)	—	—	47 ± 12
Current % of ideal body weight	125 ± 24	138 ± 24	134 ± 27
Maximum % of ideal body weight	133 ± 27	151 ± 32	154 ± 35
Treatment with insulin (%)	—	—	39
Treatment with oral agents (%)	—	—	30
On diet or without treatment	—	—	31

Data are *n*, %, or means ± SD.

TABLE 3
Affected sib-pair analysis

Marker	Heterozygosity of marker	All families			Older families		
			Alleles shared			Alleles shared	
		Number of pairs	IBD (%)	*P* value	Number of pairs	IBD (%)	*P* value
D20S107	0.80	190	50.9	0.36	96	0.526	0.24
ADA8PR	0.80	205	54.8	0.03	97	0.617	0.0006
D20S119	0.82	222	56.2	0.004	110	0.636	0.00003
D20S178	0.76	223	56.3	0.004	110	0.650	<0.00001
D20S197	0.83	221	55.7	0.009	108	0.637	0.00003
D20S176	0.63	198	52.4	0.17	95	0.593	0.006
D20S196	0.81	200	53.2	0.10	93	0.607	0.002
D20S100	0.76	176	55.3	0.02	74	0.608	0.004

Multipoint linkage analysis was used to incorporate information from all chromosome 20 markers that were evaluated in the MODY1 region to infer more effectively the probability distribution of IBD status at each point in the interval of interest (Fig. 1). Using multipoint analysis in all families, the peak NPL scores occur for markers D20S178 and D20S197 (NPL score, 1.9; $P < 0.05$). The evidence for linkage became stronger in older-onset families for the same markers (NPL score, 3.3; $P = 0.009$) and weaker in younger-onset families (NPL score, –0.20; NS).

Evidence for association between NIDDM and alleles at the three most strongly linked markers (D20S119, D20S178, and D20S197) was determined with the TDT. Using the TDT, there was no significant evidence for deviation of transmitted alleles from that expected at the marker D20S119 and D20S178. Some evidence for deviation of transmitted alleles from that expected was observed for marker D20S197. The results are presented in Table 4. In the total group, allele "E" was more frequently transmitted from heterozygous parents to NIDDM offspring ($P = 0.14$). This deviation became significant in families with an older age at diagnosis of NIDDM ($P < 0.01$). There was no deviation in the transmission of this allele in younger families (data not shown).

DISCUSSION

In this study, highly informative markers on chromosome 20q were used to genotype a set of 29 extended Caucasian families with NIDDM to test the hypothesis that the MODY1 locus or other genes in that region contribute to predisposition to the development of NIDDM. We obtained findings that support linkage between the MODY1 region (but not the MODY1 locus itself) and NIDDM, specifically in families with diabetes developing in the sixth decade of life. The results were consistent across various approaches to the analysis of the genetic data.

In contrast to the studies that failed to find linkage of NIDDM with the MODY1 region (9–11), we studied extended pedigrees in which NIDDM appeared to be transmitted in a manner consistent with an autosomal dominant disorder. We believe that this strategy improved our opportunity of detecting linkage. First, selection of large families with a similar pattern of inheritance minimized genetic heterogeneity of NIDDM. Second, data from large multigenerational families allowed more precise determination of allele phase and provided an opportunity to employ multiple analytic approaches for testing the existence of linkage to selected genetic markers. Finally, in contrast to several previous studies that used the ADA locus as a marker, we examined the entire MODY1 region. Had other studies (9–11) used markers telomeric to the ADA locus, it is possible that they too might have obtained positive findings. Consistent with this possibility, we observed only weak evidence for linkage between the ADA locus and NIDDM in our families, whereas linkage was strong with markers telomeric to the ADA locus. Results consistent with our findings have been reported recently by others (29,30).

With the transmission disequilibrium test, we found that a specific allele at the D20S197 marker was transmitted more frequently than expected from heterozygous parents to NIDDM offspring. This would suggest that the putative gene that contributes to the development of NIDDM in these older families is located in close proximity to the D20S197 marker. According to the recent integrated maps of human chromosome 20, there are no apparent known

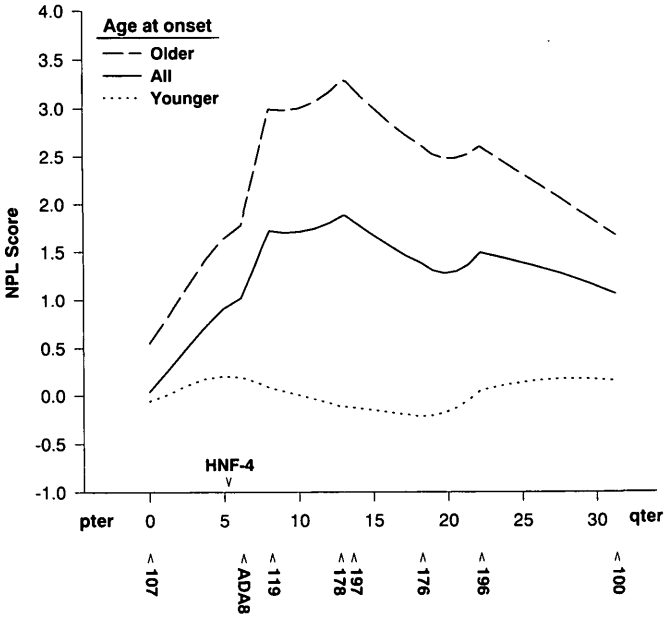

FIG. 1. Results of multipoint nonparametric linkage analysis of NIDDM with markers in the MODY1 region of chromosome 20q. Results were obtained with GENEHUNTER software (26). On the x-axis, the genetic distance between markers is presented in centimorgans. The NPL score was maximal at the marker D20S197 and corresponded to *P* values of 0.04 and 0.009 in all families and older families, respectively.

TABLE 4
TDT: transmission of alleles of marker D20S197

D20S197 allele	Size (kb)	All families Transmitted Yes	All families Transmitted No	All families *P* value	Older families Transmitted Yes	Older families Transmitted No	Older families *P* value
E	0.195	47	36	0.14	30	14	<0.01
C	0.191	44	46	0.54	28	26	0.45
F	0.201	12	12	0.50	11	10	0.50
A	0.187	27	29	0.55	10	15	0.79
B	0.189	18	17	0.50	5	11	0.89
H	0.205	10	18	0.91	3	7	0.83
I	0.207	8	9	0.50	3	8	0.89

candidate genes for NIDDM in the vicinity of the D20S197 marker (23,31).

The locus of HNF-4, the recently identified MODY1 gene (6), is ~8 cM toward the centromere from markers D20S178 and D20S197 (Fig. 1). Therefore, the TDT results for the D20S197 marker and the location of the peak NPL score around markers D20S178 and D20S197 both argue against HNF-4 as a candidate gene for the putative NIDDM locus that we have identified in the present study. Furthermore, the evidence for linkage between NIDDM and the ADA8 marker, the marker closest to HNF-4 locus, was consistently weak in our families.

At the phenotypic level, the subgroup of linked families in this study differ markedly from the RW family in which a mutation has been identified in the MODY1 gene. Diabetes in that family seems to be diagnosed in the second and third decade of life and is associated with a primary defect in insulin secretion (32,33). By contrast, affected individuals in this study group have not only an older age-at-diagnosis distribution, but also manifest no evidence of a defect in insulin secretion. They had been very obese in the past and were still obese at the time of examination.

In summary, the results of this study suggest that, in a significant proportion of NIDDM families, diabetes is linked with the region on chromosome 20q that contains the MODY1 locus (23). However, on the basis of the linkage data and the clinical characteristics of diabetes in these families, we conclude that the MODY1 gene (HNF-4) is unlikely to be the gene responsible for their susceptibility to NIDDM. The most likely interpretation of the data on these families is that the "MODY1 region" on chromosome 20q also contains a new susceptibility locus for NIDDM.

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