Further Evidence for a Susceptibility Locus for Type 2 Diabetes on Chromosome 20q13.1–q13.2

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We previously reported suggestive linkage between type 2 diabetes and markers in a region on chromosome 20q using data from a collection of 29 Caucasian families in which type 2 diabetes with middle-age–onset was segregated as an autosomal-dominant disorder. To map more precisely the susceptibility locus (or loci) within this broad region, we increased the family collection and genotyped all families for additional markers, both within the critical region and spaced over the rest of chromosome 20. Altogether 526 individuals (including 241 with diabetes) from the total collection of 43 families were included in the study. All individuals were genotyped for 23 highly polymorphic markers. Positive evidence for linkage was found for a 10-cM region on the long arm of chromosome 20q13.1–q13.2 between markers D20S119 and D20S428. The strongest evidence in two-point as well as multipoint linkage analysis ($P = 1.8 \times 10^{-5}$) occurred at the position corresponding to marker D20S196. The individuals with diabetes in the seven most strongly linked families had high serum insulin levels during fasting and 2-h post–glucose load periods. We did not find any evidence for linkage between type 2 diabetes and any other region on chromosome 20. In conclusion, our larger and more comprehensive study showed very strong evidence for a susceptibility gene for insulin-resistant type 2 diabetes located on the long arm of chromosome 20 around marker D20S196. *Diabetes* 49:2212–2216, 2000

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IBD, identical-by-descent; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; NPL, nonparametric linkage; PCR, polymerase chain reaction.

The development of type 2 diabetes is strongly influenced by genetic factors (1,2). Recently, genes responsible for several rare forms of autosomal-dominant early-onset type 2 diabetes, also known as maturity-onset diabetes of the young (MODY), have been identified (3,4). Although mutations in these genes are infrequent, their identification revealed new pathways involved in insulin secretion. The effort to find susceptibility loci for common middle-age–onset forms of type 2 diabetes is intense, but it has not yet resulted in the identification of any specific gene (5–9). Previously, we reported suggestive evidence for linkage between several markers on chromosome 20q13.1–q13.2 and the segregation of middle-age–onset type 2 diabetes as an autosomal-dominant disorder in our collection of Caucasian families with type 2 diabetes (10). We excluded hepatocyte nuclear factor-4α, an obvious candidate gene in this region, as the locus responsible for the linkage (11); findings from other family collections in which evidence for linkage with type 2 diabetes on chromosome 20q has been found have confirmed this conclusion (12–15).

To narrow the 20q chromosomal region in which a type 2 diabetes susceptibility locus may be located, we increased the number of middle-age–onset type 2 families and genotyped them for markers previously showing linkage. We also genotyped all families for additional markers in the critical region to increase the genetic information for the determination of shared identical-by-descent (IBD) markers between affected relatives. Markers spaced over the rest of chromosome 20 were genotyped to test whether there are additional regions linked with type 2 diabetes, as recently suggested (14).

The ascertainment and examination of families with type 2 diabetes have been described previously (10). For the current report, the families from the previous publication have been expanded, and the diabetes status of individuals with impaired glucose tolerance (IGT) has been updated. Moreover, 14 newly collected families have been added to the previously described set of 29 families (10). Altogether, 526 individuals from the total collection of 43 families are included in the present study: 241 individuals with diabetes, 19 with IGT, and 266 with normal glucose tolerance. On average, there are 12 individuals per family (6 with and 6 without diabetes). The mean age at diagnosis of diabetes was 45 ± 17 years. The mean age at enrollment into the study was 59 ± 14 years for individuals with diabetes and 49 ± 18 years for

TABLE 1
Description of the markers used for genotyping and the results of two-point linkage analysis

Marker name	Distances between markers (cM* or Mb†)	Position on map (cM or Mb)	NPL score	*P*	Information
D20S103	—	2.1	0.26	0.37113	0.43
D20S482	10.0	12.1	–1.07	0.86401	0.53
D20S192	6.7	18.8	–0.36	0.62129	0.49
D20S851	5.9	24.7	0.09	0.43557	0.53
D20S894	5.9	30.6	–0.02	0.48340	0.49
D20S898	4.9	35.5	1.15	0.12656	0.49
D20S912	11.2	46.7	0.31	0.35076	0.56
D20S477	0.8	47.5	–0.35	0.61568	0.46
D20S107	8.2	55.7	0.40	0.31946	0.56
D20S96†	2.8	58.5	–0.48	0.66910	0.60
D20S119†	1.1	59.6	0.64	0.24424	0.54
D20S481†	0.3	59.9	3.06	0.00427	0.54
D20S836†	1.6	61.5	0.85	0.18955	0.60
D20S197†	1.4	62.9	2.06	0.02912	0.54
D20S178†	0.7	63.6	1.94	0.03628	0.58
D20S176†	1.0	64.6	1.35	0.09355	0.40
D20S109	2.2	66.8	3.05	0.00440	0.63
D20S196	0.5	67.3	4.71	0.00010	0.61
D20S428	2.7	70.0	0.04	0.45667	0.48
D20S480	2.2	72.2	0.67	0.23622	0.50
D20S120	3.6	75.8	1.15	0.12610	0.55
D20S100	1.3	77.1	0.52	0.28091	0.50
D20S171	10.9	88.0	–0.04	0.49041	0.54

Markers were genotyped with a fluorescent method, except for D20S119, D20S178, D20S176, D20S196, and D20S100, which were genotyped with radioactive methods. *Distances between markers based on the Marshfield genetic map of chromosome 20; †distances in the interval D20S96–D20S176 are based on a physical map (16).

those without diabetes. Family members with diabetes were also more obese (percent ideal body weight 138 ± 31) than those without diabetes (128 ± 26).

All individuals were genotyped for 23 highly polymorphic markers (including the 7 reported previously) covering chromosome 20 (Table 1 and Fig. 1). The chromosomal region previously showing evidence for linkage was covered with additional markers so that the intermarker distances were all <3 Mb and, in the majority of instances, <2 Mb (Table 1). Distances between markers in the critical region were based on the radiation hybrid map recently published by us (16) and on the Marshfield sex-averaged genetic map (http://www.marshmed.org/genetics/) for the rest of chromosome 20. Linkage analysis was performed using Genehunter Plus com-

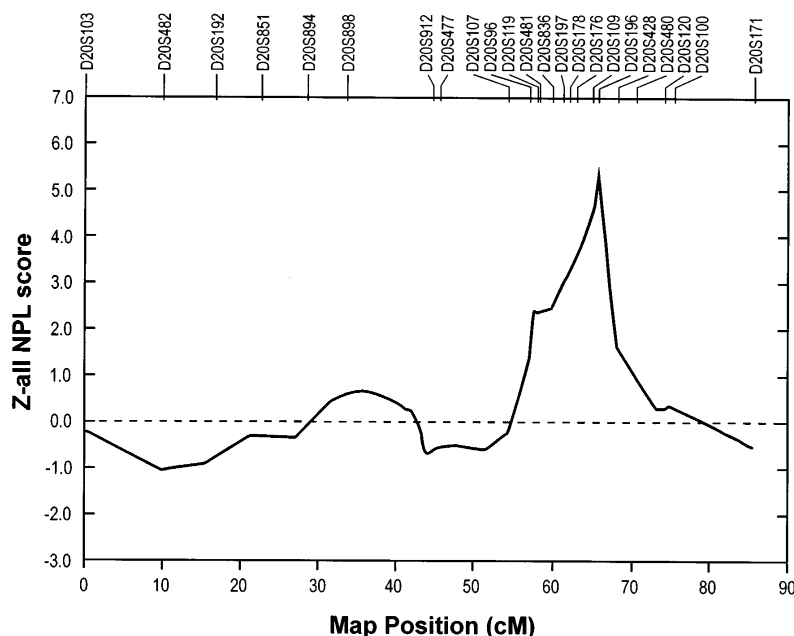

FIG. 1. Results of multipoint NPL analysis of type 2 diabetes with markers on chromosome 20. Results were obtained with Genehunters Plus (17). The names of the markers used for genotyping in this study are listed at the top of the graph. At the bottom of the graph, the position on the map is indicated in centiMorgans based on a genetic map, although distances between markers D20S96 and D20S176 are defined from a physical map (Table 1). The NPL *Z* score was maximal at marker D20S196 and corresponded to a *P* value of 1.8×10^{-5} in the entire set of families.

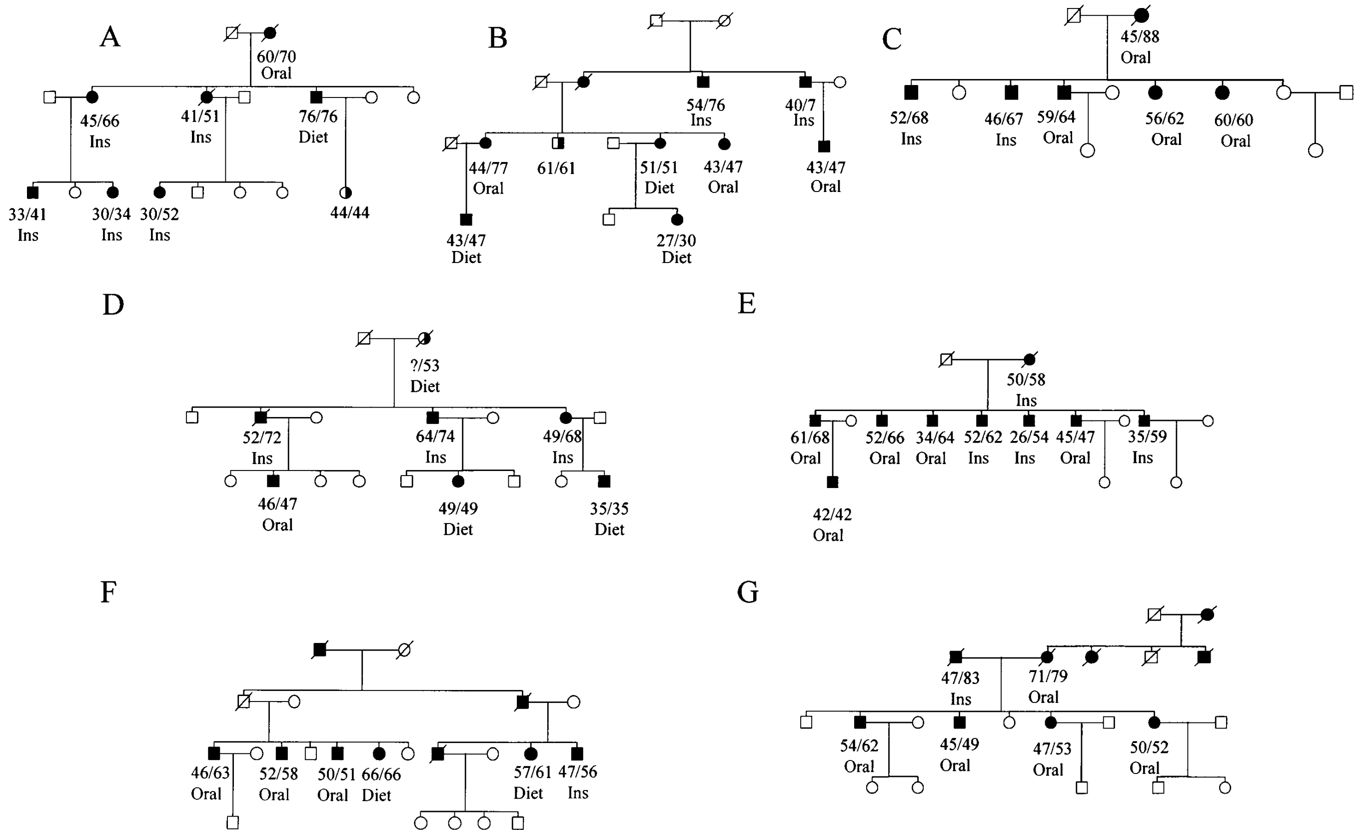

FIG. 2. Pedigrees and clinical characteristics of the seven families (*A–G*, respectively) linked to chromosome 20q. Closed, half-closed, and open symbols represent subjects with type 2 diabetes, IGT, and normal glucose tolerance, respectively. Data for the examined individuals with diabetes or IGT and data for deceased diabetic patients are shown under the symbols. Data for individuals with normal glucose tolerance are not presented. The age at diabetes diagnosis and age at examination (or death for deceased diabetics) are listed on the first line. The type of treatment for diabetes at the time of examination is listed on the second line.

puter software (17) and affection status that included diabetes together with IGT. Based on the availability of many relationships in these large pedigrees, the *Z* test was used for the analysis.

The results of two-point linkage analysis are presented in Table 1. The strongest evidence for linkage was found with marker D20S196 ($P = 0.0001$). Several closely located markers (D20S109, D20S178, D20S197, and D20S481) also showed evidence of linkage. Markers in other parts of chromosome 20 did not show evidence of linkage with type 2 diabetes.

In multipoint linkage analysis, positive evidence for linkage ($Z > 2.1$, $P < 0.01$) was found for the 10-Mb region on the long arm between markers D20S119 and D20S428. The highest *Z* score, 5.32, occurred at the position corresponding to marker D20S196 (Fig. 1), and the *P* value of 1.8×10^{-5} for this marker surpassed the criterion for strong evidence for linkage proposed by Lander and Krugliak (18). In an analysis in which patients with IGT were considered to have unknown affection status, the *Z* score was 5.19. We did not find evidence for linkage with type 2 diabetes on the short arm of chromosome 20. The evidence for linkage at marker D20S196 did not change when we used different genetic maps for chromosome 20 in which the distances between markers varied, but the marker order remained the same (data not shown)

In our family collection, 16 families had nonparametric linkage (NPL) scores >1.0 at marker D20S196, and in 7 families, the scores ranged from 2.8 to 9.5. Figure 2 presents the pedigrees of these families with selected clinical data. Transmission of type 2 diabetes was unilineal in all but one family, and three generations or more were affected by diabetes in all but one family. To determine whether diabetes in these seven families is typical for type 2 diabetes, we compared the clinical features of the 43 affected individuals (41 with diabetes and 2 with IGT) in the linked families with those of diabetic individuals in the remaining 36 families (Table 2). In regard to age at diagnosis of diabetes, various measures of obesity, and treatment for diabetes, the 43 affected members of the families with diabetes linked to chromosome 20q are very similar to the diabetic members of the other families. The high proportion of insulin-treated individuals in both groups reflects the long duration of diabetes. The two groups were also similar in regard to the levels of plasma glucose and serum insulin while fasting and 2 h after a glucose challenge. The very high insulin levels, also measured while fasting and 2 h after a glucose challenge, are noteworthy. They point to insulin resistance as a characteristic of the diabetes in both linked and unlinked families. Thus, the diabetes in the linked families does not appear to be another kind of MODY associated with low serum insulin levels (3,4).

The genetic basis of type 2 diabetes is apparently heterogeneous across populations, as indicated by conflicting results from ongoing studies (5–9,12,14,15). Only a few of these studies found suggestive evidence for linkage of type 2 diabetes to markers on chromosome 20 (12,14,15). Our study, carried out

in specially ascertained large families with type 2 diabetes, is the first to provide strong evidence for linkage of diabetes with markers on the long arm of chromosome 20q13.1–q13.2. In contrast, the results of our study do not provide any support for linkage between type 2 diabetes and markers on the short arm of chromosome 20, a finding recently reported by the Finland-U.S. Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) investigators (14).

In our previous report, the evidence for linkage was found only in a stratified analysis; spanned a very broad region that included markers D20S119, D20S197, DS20S178, and D20S196; and reached a level that was only suggestive of linkage (10). The present report is based on a larger number of families, a larger number of genetic markers, and a more accurate map of chromosome 20. Our new results are in agreement with the previous report, but they also provide much stronger evidence for linkage and a more precise localization of the putative type 2 diabetes locus, the region around marker D20S196 ($P = 1.8 \times 10^{-5}$). Several other studies have also found evidence for linkage with chromosome 20q in Caucasians. Suggestive evidence for linkage with markers D20S197 and D20S178 was found in the FUSION study, which examined a large collection of Finnish sib pairs with type 2 diabetes (14). According to our radiation hybrid map, these two markers are located ~3.7 Mb centromeric from D20S196. Weak evidence for linkage at the same location was also reported by Bowden et al. (15) in a small collection of sib pairs. Interestingly, a French study of a small number of sib pairs with early-onset type 2 diabetes found suggestive evidence of a susceptibility locus in the PCK1 region of chromosome 20q, a region that includes D20S196 (12).

At least two potential candidate genes for type 2 diabetes are localized to the region between marker D20S197 and D20S196: protein tyrosine phosphatase-B, a negative regulator of insulin signaling that may influence insulin sensitivity (19), and CAAT/enhancer-binding-protein β, which is an inhibitor of insulin gene transcription (20). These genes need to be examined for DNA sequence differences that segregate with diabetes in the linked families. In addition, transcript maps for this region need to be developed so new candidate genes can be identified. In conclusion, this report presents further evidence that chromosome 20q may contain a novel susceptibility locus for type 2 diabetes associated with insulin resistance.

RESEARCH DESIGN AND METHODS

Families. The families used in this study were recruited for research on the genetics of type 2 diabetes at the Joslin Diabetes Center. Details of the recruitment of the first set of 29 families have been published previously (10). During the last 2 years, additional affected individuals in these families were recruited and those with IGT were reexamined. In addition, 14 new families were recruited. The following is a brief description of the selection and examination of the families. We sought to identify families with a pattern of occurrence of type 2 diabetes that was consistent with an autosomal-dominant mode of inheritance. An additional selection criterion was the availability of a large number of family members (with and without diabetes) willing to participate in the study. The screening criteria to identify eligible families consisted of the following: *1*) an index case and at least one sibling had type 2 diabetes diagnosed between ages 35 and 59 years, *2*) the index case had been treated for at least 2 years with diet or oral agents, and *3*) diabetes occurred in the family in at least three generations. Index cases were selected from the Joslin Clinic population, and only families of European origin were used in the present study. The Committee on Human Subjects of the Joslin Diabetes Center approved the study protocol and informed consent procedures. After written consent to participate was obtained, individual family members were examined. Fasting blood was drawn for blood glucose determination, DNA extraction, and other biochemical measurements (including serum insulin). Nondiabetic and diabetic individuals treated with oral agents or diet had an additional blood sample drawn 2 h after an oral challenge with 75 g glucose. Height, weight, and blood pressure were also measured. All participants completed medical and family history questionnaires that were supplemented by information abstracted from medical records. A description of the methods to measure blood glucose and serum insulin levels was reported previously (10). The percent of ideal body weight was calculated as BMI × 4.39 for males and as BMI × 4.76 for females (21). Diabetes and IGT were diagnosed according to World Health Organization criteria (22).

Genotyping. All individuals were genotyped for 23 highly polymorphic markers (including the 7 reported previously) covering chromosome 20 (Fig. 1 and Table 1). The chromosomal region previously showing evidence for linkage was covered with additional markers, so the intermarker distances were <3 Mb and, in the majority of instances, <2 Mb (Fig. 1 and Table 1). Distances between markers in the critical region were based on our recently published radiation hybrid map (16) and on the Marshfield sex-averaged genetic map (http://www.marshmed.org/genetics/) for the rest of chromosome 20. Because of difficulties in genotyping marker ADA8, which had been used in our previous study (10), we did not use it in the present study. According to our radiation hybrid map, ADA8 is located within a 1.1-Mb interval between D20S96 and D20S119, so its omission has little impact on the results. Also, we found that the order of the markers D20S197 and D20S178 are reversed from the order used previously. The position of D20S178 is actually 0.7 Mb telomeric, rather than centromeric, of D20S197 (16).

All individuals were genotyped using a fluorescent method for the following markers: D20S103, D20S482, D20S192, D20S851, D20S894, D20S898, D20S912, D20S477, D20S107, D20S96, D20S481, D20S836, D20S197, D20S109, D20S428, D20S480, D20S120, and D20S171. The forward primers were labeled with the dyes TET, FAM, or HEX (Research Genetics, Huntsville, AL). Polymerase chain reaction (PCR) was performed in a 15-µl volume in a 96-well PCR plate (MJ Research, Watertown, MA). Each reaction contained 50 ng genomic DNA, 4 pmol of each primer, 25 µmol/l dNTPs, 1–2 mmol/l $MgCl_2$, 3 µl of 5 × PCR buffer, and 0.2 U Amplitaq Polymerase (PerkinElmer, Foster City, CA). The PCR program for all markers was as follows: 5-min hot start at 95°C, Taq added, and 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 30 s, followed by 10 min at 72°C, and held at 4°C. All reactions were carried out in 96-well PCR machines (MJ Research). The electrophoresis was performed on the ABI 377 DNA automatic sequencer (PE-ABI, Foster City, CA). Once the data were collected, they were analyzed by two different observers using GeneScan and Genotyper software (PE-ABI). For the other five markers (D20S119, D20S178, D20S176, D20S196, and D20S100), the previously described radioactive method of genotyping was used (10).

Genetic analysis. For the linkage analysis, distances between the markers were based on the radiation hybrid map developed by us for the region linked

TABLE 2
Comparison of clinical characteristics of individuals with diabetes in study families according to whether their family is linked with chromosome 20q

	Chromosome 20 q–linked families	Remaining families
Families	7	36
Subjects (M/F)	27/16	106/112
Age at diagnosis (years)	46 ± 14	45 ± 18
Age at examination (years)	56 ± 11	60 ± 14
% IBW at examination	136 ± 36	139 ± 29
Lifetime maximum % IBW	156 ± 43	153 ± 31
% Treated with diet alone	22.0	16.8
% Treated with oral agents	43.9	38.4
% Treated with insulin	34.1	44.8
Non–insulin-treated diabetic patients		
Fasting blood glucose (mg/dl)	174 ± 75	170 ± 80
2-h Blood glucose (mg/dl)	272 ± 132	245 ± 76
Fasting serum insulin (µU/ml)	18.7 ± 10.8	15.1 ± 9.0
2-h Serum insulin (µU/ml)	55.6 ± 41.2	51.6 ± 73.1

Data are *n* or means ± SD, unless otherwise indicated. IBW, ideal body weight.

with type 2 diabetes (16) and on the Marshfield sex-averaged genetic map for the rest of the chromosome 20 (Table 1 and Fig. 1). Allelic frequencies of markers were estimated from 80 unrelated nondiabetic individuals (i.e., spouses of type 2 diabetes patients). Model-independent multipoint linkage analysis was performed using the Genehunter Plus program (17), which considers marker allele sharing between all pairs of affected family members. The nonparametric logarithm of odds (LOD) score (the NPL Z score) for two-point and multipoint analysis was estimated at each position on the map by comparing the observed IBD sharing among affected family members with that expected under the null hypothesis of no linkage. The P values for Z scores were determined from the large-sample theory for a normal deviate (17). Families with a maximal Z score >2.1 in the region between markers D20S119 and D20S428 were considered to be linked to chromosome 20q12–q13.1.

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