

Identification of a Locus for Maturity-Onset Diabetes of the Young on Chromosome 8p23

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Maturity-onset diabetes of the young (MODY) is a subtype of diabetes defined by an autosomal dominant inheritance and a young onset. Six MODY genes have been discovered to date. To identify additional MODY loci, we conducted a genome scan in 21 extended U.S. families (15 white and 6 from minorities, for a total of 237 individuals) in which MODY was not caused by known MODY genes. Seven chromosomal regions (1q42, 2q24, 2q37, 4p13, 8p23, 11p15, and 19q12) had a parametric heterogeneity logarithm of odds (HLOD) ≥ 1.00 or a nonparametric logarithm of odds (LOD) ≥ 0.59 ($P \leq 0.05$) in the initial screen. After typing additional markers at these loci to reduce the spacing to 2–3 cM, significant linkage was detected on 8p23 (HLOD = 3.37 at D8S1130 and nonparametric LOD = 3.66; $P = 2 \times 10^{-5}$ at D8S265), where a 4.7-Mb inversion polymorphism is located. Thirty percent of the families (6 of 21) were linked with this region. Another linkage peak on chromosome 2q37 with an HLOD of 1.96 at D2S345/D2S2968 accounted for diabetes in an additional 25% of families (5 of 21). All 6 minority families were among the 11 families linked to these loci. None of the other loci followed up had an HLOD exceeding 1.50. In summary, we have identified a MODY locus on 8p23 that accounts for diabetes in a substantial proportion of MODY cases unlinked to known MODY genes. Another novel MODY locus may be present on 2q37. Cloning these new MODY genes may offer insights to disease pathways that are relevant to the cause of common type 2 diabetes. *Diabetes* 53:1375–1384, 2004

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BAC, bacterial artificial chromosome; CEPH, Centre d'Etude du Polymorphisme Humain; FISH, fluorescence in situ hybridization; GCK, glucokinase; HLOD, heterogeneity logarithm of odds; HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; MODYX, unaccounted MODY.

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Maturity-onset diabetes of the young (MODY) is a subtype of diabetes defined by an autosomal dominant pattern of inheritance and a young onset, often before age 25 (1). MODY may account for up to 5% of cases of type 2 diabetes. The availability of large families with multiple affected members has facilitated studies of this form of diabetes. Six distinct MODY genes have been identified in different sets of pedigrees and populations. The first MODY gene to be recognized was glucokinase (*GCK*) (2,3), followed by hepatocyte nuclear factors HNF-1 α (*TCF1*) and HNF-4 α (*HNF4A*) (4,5). GCK is the enzyme responsible for the initial processing of glucose in the β -cell, whereas HNF-1 α and HNF-4 α are transcription factors that modulate the expression of several genes involved in the differentiation and function of β -cells (6). Mutations in another three transcription factors are associated with rarer forms of MODY, namely insulin promoter factor 1 (7), HNF-1 β (*TCF2*) (8), and neurogenic differentiation 1 (9). These forms of diabetes are typically characterized by an insulin secretory defect (6).

Several reports suggest that other MODY genes exist in addition to the six identified to date. In France and England, ~25% of MODY pedigrees do not show linkage with GCK, HNF-1 α , or HNF-4 α (10,11). The proportion of unaccounted MODY (MODYX) is even higher, up to 50–60%, if one takes into account that MODY is often diagnosed after the traditional age limit of 25 years (12). As has been the case with other MODY genes (13–17), cloning the genes responsible for these other forms of MODY may lead to the discovery of disease pathways that are also relevant to more common varieties of type 2 diabetes.

Here we report the results of an autosomal genome scan of 21 extended MODYX families from the U.S. population. Our data provide significant evidence for a MODY locus on 8p23 that accounts for diabetes in 30% of our MODYX families. Another MODYX gene, accounting for some of the remaining families, may be located on 2q37.

RESEARCH DESIGN AND METHODS

Families. Twenty-one extended families with early-onset, autosomal dominant type 2 diabetes not caused by known MODY genes (MODYX) were included in this study. These families belong to the Joslin Study on Genetics of Type 2 Diabetes, a collection of 104 extended families in which type 2 diabetes segregates as an autosomal dominant disorder (12). The screening criteria used for the Joslin family collection were 1) a proband and at least one sibling with type 2 diabetes diagnosed between ages 10 and 59 years, 2) three or more generations affected by diabetes, and 3) unilineal transmission of

TABLE 1
Clinical and family-structure characteristics of the MODYX families

Total families (<i>n</i>)	21*
Total individuals (<i>n</i>)	237
Affected individuals (<i>n</i>)	133
Age at diagnosis (years)	31 ± 18†
IGT/gestational diabetes (%)	12.2
IBW (%)	132 ± 30
Treatment	
Diet only (%)	25.9
Oral agents (%)	22.3
Insulin (%)	51.8

IBW, ideal body weight. *There were six nonwhite families: three black, two Hispanic, and one Pacific Islander. †Data are means ± SD.

diabetes. Diabetes in a proband was considered non-insulin-dependent when hyperglycemia was managed without insulin for at least 2 years after diagnosis. An additional selection criterion was the availability of a large number of family members (with and without diabetes) who were willing to participate in the study. The 21 families included in the present study were those in the collection that had an average age at diagnosis of 35 years or younger and were not linked to known MODY genes (12). The 35-year cutoff was supported by our previous findings on MODY3, showing that families who carry HNF-1 α mutations have an average age at diabetes diagnosis ranging from 11 to 31 years (mean 21 years) (12). Of the 21 families included in the present study, 15 were white, 3 were black, 2 were Hispanic, and 1 was Pacific Islander. The four Joslin families that we previously described as young-onset families linked to 12q15 (18) have an average age at diagnosis >35 years and were not part of this study.

Phenotype definition. Diabetes was defined as one of the following: 1) treatment with insulin or oral agents and the presence of diabetic hyperglycemia confirmed by the study examination, 2) oral glucose tolerance test blood glucose values meeting World Health Organization criteria (fasting ≥ 140 mg/dl or 2 h ≥ 200 mg/dl), or 3) HbA_{1c} $\geq 7.0\%$ (normal values <6.1%) in individuals who declined the oral glucose tolerance test or were not fasting when examined (19,20). When glucose values exceeded the World Health Organization criteria for normal glucose tolerance but did not meet any of these criteria, a diagnosis of impaired glucose tolerance (IGT) was made. A woman with normal glucose values at examination but a documented history of diabetes during pregnancy was classified as having previous gestational diabetes.

Characteristics of family members. The 21 MODYX pedigrees included a total of 237 family members, 133 of whom had diabetes, IGT, or previous gestational diabetes (based on medical history). Salient clinical characteristics of these individuals are shown in Table 1. On average, each family included seven examined members with diabetes, IGT, or previous gestational diabetes and five nonaffected individuals. The average age at diagnosis in affected members was 31 years. Each family included at least one individual whose diabetes was diagnosed before age 25, although this was not used as a screening criterion.

Initial genome-wide scan. Genomic DNA was prepared from peripheral blood by phenol-chloroform extraction. A genome scan was performed by the National Heart, Lung, and Blood Institute Mammalian Genotyping Service at the Marshfield Medical Research Foundation by means of PCR and automated fragment analysis (21). Each individual was genotyped for 377 autosomal microsatellite markers with an average heterozygosity of 0.76 (Marshfield Set 10), resulting in a mean distance between markers of 9.3 cM. Information on the screening set and genotyping protocols is available at the Marshfield web site (<http://research.marshfieldclinic.org/genetics>). The proportion of markers that were genotyped successfully for each individual ranged from 89.4 to 99.8%, with 84% of the participants having information on >97% of the markers. The overall error rate, as measured on replicate Centre d'Etude du Polymorphisme Humain (CEPH) samples, was 0.5%.

Follow-up genotyping. After the initial genome scan, five chromosomal regions were followed by genotyping 41 additional microsatellite markers, to increase the average information content at each location to 0.80 or higher. Each of the 41 markers was amplified individually by fluorescence-labeled PCR and sized by means of capillary gel electrophoresis using an ABI 310 Genetic Analyzer and the GENESCAN 3.1.2 and GENOTYPER 2.5 software (Applied Biosystems). Genotypes were read independently by two researchers, and ambiguous results were repeated.

Genotypes from the initial genome screen were analyzed by means of the

program PREST (22) to check the accuracy of relationships between individuals within families, and adjustments of the family structures were made accordingly. The programs PEDCHECK (23) and SIMWALK2 (24) were then used to detect genotyping errors and inconsistencies. When the mistyping was not resolved by further review of the gel images, the genotypes were set to be unknown.

Linkage analysis. Allele frequencies were estimated at each marker locus on the basis of the genotype distributions in the entire dataset. Marker order and intermarker distances (sex averaged) were those specified in the Marshfield map (http://research.marshfieldclinic.org/genetics/Map_Markers/mapmaker/MapFormFrames.html).

Two-point and multipoint linkage analyses were performed using GENEHUNTER-PLUS (25). Analyses were performed twice, with the disease status for individuals with IGT and gestational diabetes set either to "affected" or to "unknown." In addition to nonparametric methods, evidence for linkage was evaluated by parametric methods, because these are more powerful when there is apparent Mendelian transmission with high penetrance, as is the case for diabetes in these families. Evidence for linkage assuming homogeneity (logarithm of odds [LOD]) and heterogeneity (heterogeneity LOD [HLOD]) was determined. For parametric analysis, the LOD scores were calculated assuming an autosomal dominant mode of inheritance and a disease allele frequency of 0.001, consistent with the rarity of families segregating these forms of diabetes. Similar to previous linkage analyses of MODY3 (10), four age-related liability classes (<10, 10–25, 25–40, and >40 years) were assumed, with penetrances for the homozygous and heterozygous susceptible genotypes set to 0.30, 0.50, 0.70, and 0.90, respectively. On the basis of the risk of diabetes in the general population, penetrances for the nonsusceptible genotype were set to 0.001, 0.005, 0.01, and 0.10, respectively, to allow for sporadic cases. Under the heterogeneity model, the disease was assumed to be due to mutations linked to the marker(s) in a proportion α of the families and to mutations unlinked to the marker(s) in a proportion 1- α of families. Analyses using the HLOD were used because an admixture model represents the preferred method of analysis under conditions of heterogeneity (26).

For nonparametric analyses, the S_{all} scoring statistic—a measure of allele-sharing identical-by-descent among all affected family members—was used for calculation of the nonparametric linkage Z_{all} score (27). Allele-sharing LOD scores were then derived using an exponential model, which, as discussed by Kong and Cox (25), provides a good fit to data consisting of a relatively small number of pedigrees with very extreme identical-by-descent sharing. A total of 5 affected and 23 unaffected individuals (mostly belonging to the youngest generations) from seven families were automatically eliminated from the analysis by GENEHUNTER-PLUS because of computational limitations. For accounting for the possible loss of information as a result of such pedigree trimming, the two-point parametric linkage analysis was repeated for regions of interest using the LINKAGE (28) and HOMOG (29) programs.

P values for homogeneity and allele-sharing LOD scores were generated according to the method proposed by Nyholt (30), whereas HLOD P values were estimated according to the method proposed by Abreu et al. (31), using the EXCEL conversion spreadsheet provided by Nyholt. For defining the boundaries of linked intervals through the analysis of recombinants, haplotypes were inferred in linked families by GENEHUNTER (27) and SIMWALK2 (24).

Fluorescence in situ hybridization. Dual-color fluorescence in situ hybridization (FISH) was used to determine the orientation of the 8p23 haplotype linked with diabetes in three families who showed a LOD score ≥ 1.0 at this location. Bacterial artificial chromosomes (BACs) RP11-90J21 and RP11-80B8, placed 2.5 Mb apart within the inversion polymorphisms, were used as probes. BAC 90J21 (2 μ g of DNA) was labeled with digoxigenin-11-dUTP as described in Zhao et al. (32), whereas BAC 80B8 DNA (2 μ g of DNA) was labeled with biotin-11-dUTP using the BioNick Labeling System (Invitrogen). Each BAC DNA was coprecipitated with 60 μ g of Cot-1 DNA (Invitrogen Life Technologies) and resuspended in 1 \times TE at 400 μ g/ml. Hybridization of elongated chromosomes obtained from patients' peripheral blood samples and a control lymphoblastoid cell line heterozygous for the inversion (CEPH Individual #1331-2, #GM06990C, NIGMS Human Genetic Cell Repository, Coriell Institute for Medical research, Camden, NJ) was performed according to a previously described method (33). Biotin and digoxigenin-labeled probes were detected using reagents supplied in the Oncor Kit according to the manufacturer's recommendations. Metaphase chromosomes and interphase nuclei were counterstained with 4,6-diamidino-phenylindole-dihydrochloride. Hybridization was observed with a Zeiss Axiophot microscope on 20–40 metaphases per case. Images were captured and printed using the CytoVision Imaging System (Applied Imaging, Pittsburgh, PA).

TABLE 2

Regions with multipoint parametric HLOD ≥ 1.0 or nonparametric LOD ≥ 0.59 in the initial genome scan

Chromosome	Marker*	Position (cM) [†]	Parametric HLOD	Nonparametric	
				LOD	<i>P</i>
1	D1S235	254.6	1.17	0.62	0.046
2	D2S1776	178.3	1.23	0.26	0.137
	D2S2968	251.9	1.23	0.46	0.073
4	D4S1627	60.2	0.52	0.98	0.017
8	GATA151F02	27.4 [‡]	0.83	0.87	0.023
11	D11S1984	2.1	1.03	1.21	0.009
19	D19S433	51.9	1.08	0.55	0.056

*Markers are the nearest to the peak in the Weber set 10. [†]Distance from pter, according to the Marshfield map. [‡]The peak location of chromosome 8 was 9 cM from pter in the nonparametric analysis.

RESULTS

Initial genome scan. Results of the multipoint linkage analysis of the Mammalian Genotyping Service markers ($n = 377$) are summarized in Table 2. In the initial screen, seven chromosomal regions (1q42, 2q24, 2q37, 4p13, 8p23, 11p15, and 19q12) had a parametric HLOD ≥ 1.00 or a nonparametric LOD ≥ 0.59 (approximating a global $P \leq 0.05$). Multipoint results were generally supported by two-point analyses (data not shown). Results were similar when the phenotype of individuals with IGT or gestational diabetes were set to "unknown" rather than "affected." The regions on 1q42, 2q37, 8p23, 11p15, and 19q12 were examined further by placing additional markers in each region to reduce the average spacing to 2–5 cM and simultaneously increase the information content to at least 0.80. **8p23.** Eight additional markers were added to the 8p23 region, which reduced the average spacing from 9 to 2.4 cM. A comparison between the Marshfield map and the newly released, high-resolution deCODE map (34) revealed a discrepancy in the marker order in this region, with the entire segment between markers D8S1825 and D8S1130 having opposite orientation in the two maps (Table 3). This segment corresponds to an inversion polymorphism of 4.7 Mb flanked by two highly homologous, repeated segments rich in olfactory receptor genes (35–37). Twenty-six percent of individuals of European descent have been reported to be heterozygous for the inversion (36). Because differences in marker order may affect multipoint estimates (38,39), the linkage analysis in this region was limited to individual markers (no multipoint analyses). Significant evidence of linkage was observed at D8S1130—one of the markers placed within the inversion polymorphism. The HLOD at this position was 3.37 ($P = 0.0001$), and the nonparametric allele-sharing LOD score was 2.67 ($P = 0.0002$; Table 4). Linkage was also detected at flanking markers D8S265 (HLOD = 2.38, $P = 0.001$; allele-sharing LOD = 3.66, $P = 0.00002$) and D8S1706 (HLOD = 1.58, $P = 0.008$; allele-sharing LOD = 2.94, $P = 0.0001$). For accounting for the possible loss of information as a result of the trimming of individuals by GENEHUNTER, the two-point analyses were repeated using the LINKAGE and HOMOG programs. The evidence of linkage decreased, with marker D8S1130 providing the maximum HLOD of 2.76 ($P = 0.0005$) at a recombination fraction (θ) of 0.00. The proportion of linked families (α)

was estimated to be 30%, corresponding to six pedigrees, three of white and three of black ancestry, having individual LOD scores >0.5 at $\theta = 0.00$ (Table 5). The difference in HLOD estimates obtained from GENEHUNTER and LINKAGE was due to three nonpenetrant individuals from a large, linked family. These individuals were eliminated from the analysis by GENEHUNTER because of computational limitations.

For defining the boundaries of the critical interval, the orientation of the haplotypes linked with diabetes was determined in the three families who showed the highest LOD scores at this location (F8, F9, and F15). This was accomplished by means of dual-color FISH using BAC 90J21 and BAC 80B8 as probes (Fig. 1A). The location of these BACs within the polymorphic region and their altered order in the presence of the inversion were confirmed in a control lymphoblastoid cell line known to be heterozygous for the inversion (CEPH individual #1331-2 [36]). Multiple affected members who shared the haplotype linked with diabetes but were discordant for the other haplotype were examined in each linked family. Examples of FISH analyses showing homozygosity for the inversion, heterozygosity, and homozygosity for the noninverted allele are shown in Fig. 1B–D. By analyzing the segregation of these hybridization patterns in each family, the linked haplotype was determined to be inverted in families F8 and F9 and noninverted in family F15. Obligate recombinants in F8 and F9 mapped the critical interval to the 4 cM between markers D8S1706 and D8S1721 on the "inverted" map (Fig. 2A), corresponding to ~ 2.7 Mb in the latest

TABLE 3

Comparison of the Marshfield and deCODE maps on chromosome 8p

Marker	Marshfield (cM)	deCODE (cM)	Golden path (Mb)
D8S262	4.3	6.60	3.9
D8S518	5.6	9.40	4.7
D8S1742	7.7	14.5	6.4
D8S277	8.3	15.2	6.8
D8S561	8.3	16.0	6.9
D8S1706	10.5	—	7.1

D8S1825	15.4	22.2	8.9
D8S503	16.2	—	9.2
D8S516	17.0	21.7	9.4
D8S1721	17.0	20.8	10.1
D8S376	20.6	20.0	10.9
D8S265	21.9	20.0	11.2
D8S1695	21.9	19.4	11.3
D8S1759	21.9	18.9	11.5
D8S1130	22.4	18.2	11.8

D8S552	26.4	23.6	12.6
D8S1106	27.4	23.6	12.7
D8S1754	27.4	25.2	12.9
D8S1790	27.4	25.2	13.0
GATA151F02	27.4	25.2	13.2

Dashed lines (—) represent the boundaries of the region that shows discrepancies between the Marshfield and deCODE maps. They correspond to the location of gaps in the human genome assembly. Markers in bold are those that were used in our study. D8S503 was the only marker in the inverted region to be included in the initial genome screen (Marshfield Set 10).

TABLE 4
Two-point LOD scores for nine markers on chromosome 8p in 21 families

Marker	Position (cM)*		Linkage under heterogeneity		Nonparametric LOD	
	deCODE	Marshfield	HLOD	P	LOD	P
D8S262†	6.6	4.3	0.51	0.109	0.75	0.032
D8S518	9.4	5.6	0.14	0.287	0.55	0.056
D8S1742	14.5	7.7	0.82	0.051	1.44	0.005
D8S1706	—	10.5	1.58	0.008	2.94	0.0001
D8S1130	18.2	22.4	3.37	0.0001	2.67	0.0002
D8S265	20.0	21.9	2.38	0.001	3.66	0.00002
D8S1721	20.8	17.0	0.55	0.098	0.13	0.22
D8S503†	—	16.2	0.34	0.167	0.09	0.26
GATA151F02†	25.2	27.4	1.00	0.033	0.29	0.124

*Location of markers from pter are shown in the deCODE and Marshfield genetic map, respectively. †Markers included in the initial genome screen (Marshfield Set 10).

human genome assembly. An obligate recombinant in family F15 confirmed marker D8S1706 as the telomeric boundary of the linked region (Fig. 2B).

2q37 and other regions. After 11 markers were added (average spacing 3 cM) to the 2q37 region, the evidence of linkage was increased. The maximum HLOD (1.96) was near D2S345 (251 cM; Fig. 3), in close proximity to *NIDDM1* (262 cM) in Mexican Americans with a later onset of type 2 diabetes (40). The proportion of linked families (α) was 0.23. This corresponded to five families (two white, two Hispanic, and one Pacific Islander) who had individual LOD scores ranging from 0.8 to 1.8. On the basis of recombination events in these five “linked” families, the critical interval at this location was estimated to span 8 cM from D2S2205 to D2S2253.

Twelve additional markers were added to the 19p13–19q13 region, resulting in an average spacing of 1.6 cM.

TABLE 5
Two-point LOD scores between D8S1130 and diabetes in 21 families

	Recombination fraction								
	0	0.04	0.08	0.12	0.16	0.20	0.24	0.28	0.30
F1 (W)	-1.89	-0.82	-0.55	-0.4	-0.29	-0.22	-0.16	-0.11	-0.09
F2 (W)	-2.10	-1.42	-1.11	-0.88	-0.71	-0.56	-0.43	-0.32	-0.27
F3 (W)	-1.30	-0.57	-0.30	-0.15	-0.06	0.00	0.03	0.05	0.05
F4 (B)	1.09	1.06	1.01	0.93	0.83	0.72	0.60	0.47	0.40
F5 (W)	-2.25	-1.42	-1.02	-0.77	-0.59	-0.46	-0.35	-0.26	-0.22
F6 (B)	0.61	0.61	0.59	0.54	0.49	0.43	0.37	0.31	0.29
F7 (H)	-1.58	-0.81	-0.52	-0.35	-0.23	-0.15	-0.10	-0.06	-0.05
F8 (W)	1.72	1.61	1.50	1.38	1.26	1.13	0.99	0.84	0.77
F9 (W)	1.59	1.46	1.33	1.20	1.05	0.90	0.75	0.60	0.52
F10 (H)	-2.13	-1.15	-0.82	-0.60	-0.45	-0.33	-0.24	-0.16	-0.13
F11 (P)	-1.46	-0.84	-0.58	-0.42	-0.31	-0.22	-0.16	-0.11	-0.09
F12 (W)	-1.82	-0.96	-0.69	-0.53	-0.41	-0.32	-0.25	-0.20	-0.17
F13 (W)	-1.18	-0.30	-0.08	0.02	0.08	0.11	0.12	0.12	0.11
F14 (W)	0.37	0.31	0.26	0.21	0.17	0.13	0.10	0.07	0.06
F15 (W)	1.12	1.03	0.94	0.85	0.75	0.65	0.55	0.45	0.40
F16 (W)	0.03	0.05	0.06	0.06	0.05	0.05	0.04	0.03	0.02
F17 (B)	0.77	0.71	0.64	0.58	0.51	0.44	0.37	0.30	0.26
F18 (W)	-1.31	-0.62	-0.37	-0.23	-0.14	-0.08	-0.04	-0.02	-0.01
F19 (W)	-2.40	-0.77	-0.27	0.00	0.17	0.27	0.32	0.34	0.34
F20 (W)	-4.25	-1.91	-1.18	-0.77	-0.50	-0.32	-0.19	-0.10	-0.07
F21 (W)	0.29	0.28	0.25	0.22	0.18	0.14	0.11	0.08	0.06
Total LOD	-16.06	-4.45	-0.92	0.88*	1.85*	2.31*	2.44*	2.31*	2.19*

W, white; B, black; H, Hispanic; P, Pacific Islander. *The positive homogeneity LOD scores at $\theta \geq 0.12$ are secondary to the presence of linkage heterogeneity (HLOD = 2.76) at $\theta = 0.00$.

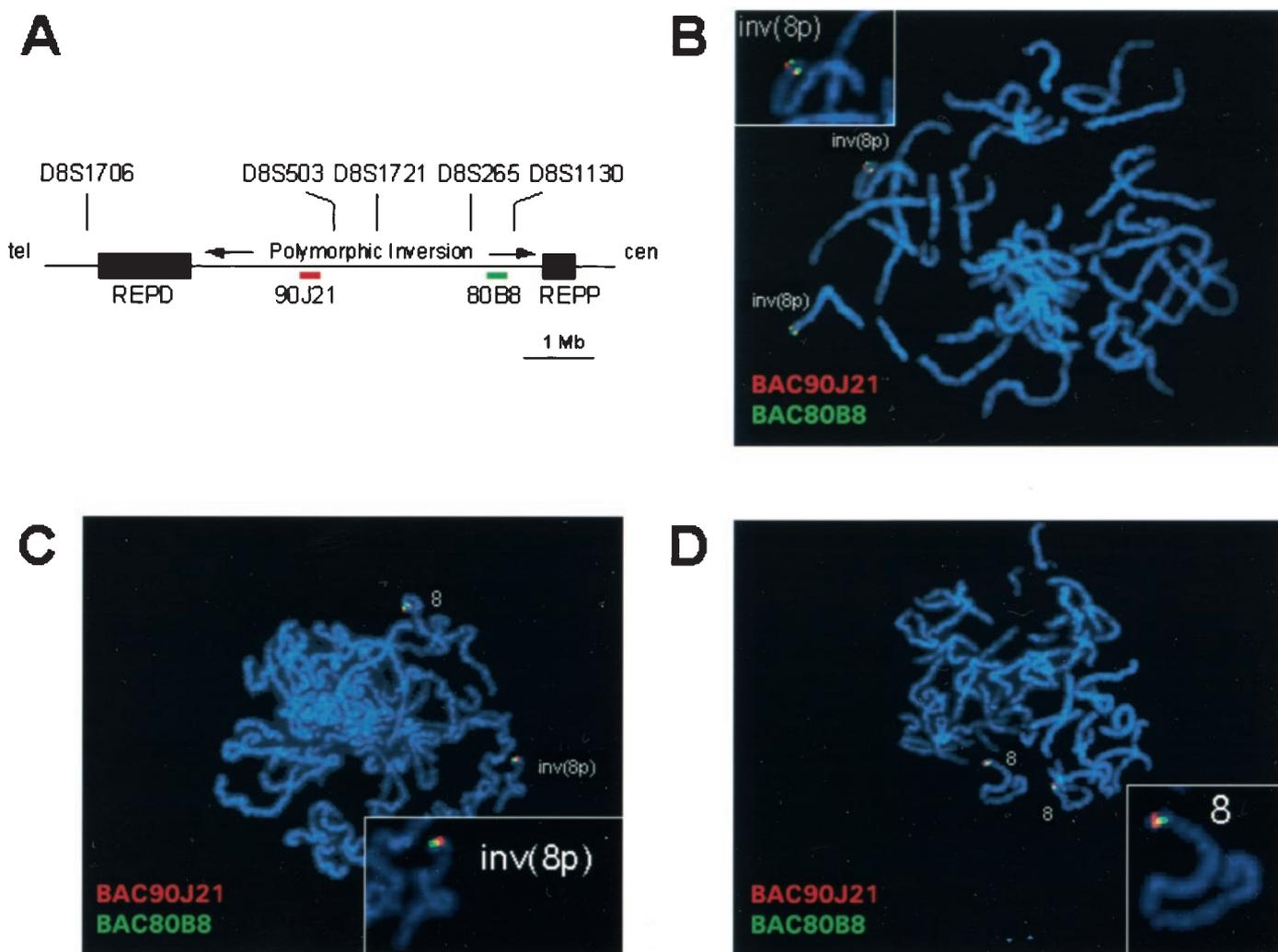


FIG. 1. Dual-color FISH of the 8p23 inversion polymorphism. **A:** Schematic representation of the 8p23 inversion polymorphism. REPD, distal repeat; REPP, proximal repeat. The vertical lines indicate the positions of the markers that were used for linkage analysis. The two BACs are represented with the colors of their labels in the FISH determination. **B:** Individual homozygous for the tel-green-red-cen hybridization pattern, consistent with an inversion on both copies of chromosome 8. **C:** Individual heterozygous for the inversion (tel-green-red-cen pattern seen on one copy of chromosome 8). **D:** Individual homozygous for the tel-red-green-cen hybridization pattern, consistent with no inversion.

with diabetes (8p23, 2q37, and all others pooled together), along with the features of 13 Joslin MODY3 families (12). As previously shown, diabetic individuals from the MODYX families were on average heavier than the MODY3 individuals ($P < 0.001$). This difference in body weight, however, was entirely accounted for by the families who were linked to 8p23 and 2q37, which had a significantly higher percentage of ideal body weight than all other MODYX families ($P = 0.004$). For the 2q37 families, the higher body weight was also present in the nondiabetic members, whereas in the 8p23 families, the higher prevalence of obesity was present only in the diabetic individuals ($P = 0.042$ vs. nondiabetic members of the same families).

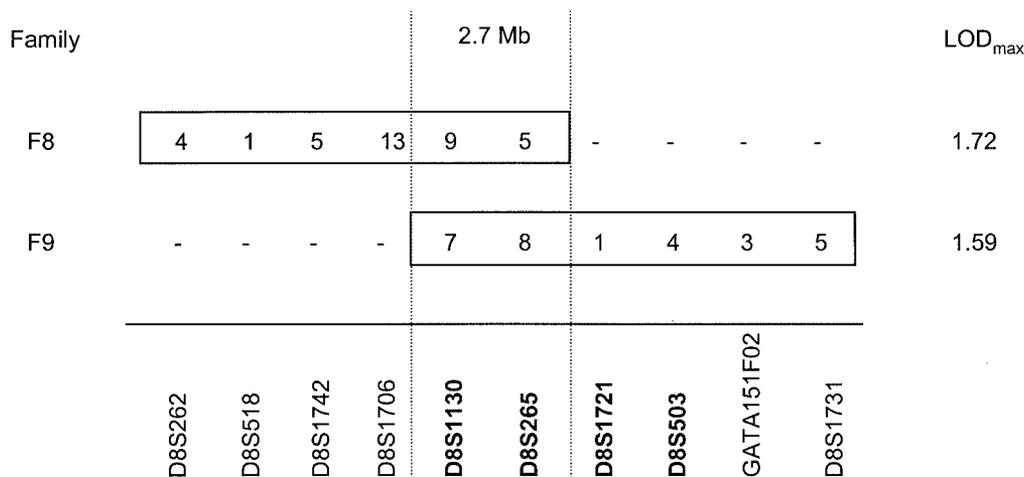
DISCUSSION

We have performed a genome-wide scan in a panel of families in which early-onset type 2 diabetes segregates as an autosomal dominant disorder and is not caused by known MODY genes. Our results point to the existence of a MODY gene on chromosome 8p23 that may account for

diabetes in 30% of these families. The results also suggest the existence of another MODY locus on chromosome 2q37, in close proximity to *NIDDM1*, that may account for diabetes in an additional 25% of families. Additional MODY loci may exist on chromosomes 1q42, 2q24, 4p13, 11p15, and 19q12, accounting for diabetes in some of the remaining families. These results add more loci to the already established genetic heterogeneity of MODY, with different MODY genes being responsible for diabetes in different groups of families.

Individuals with diabetes linked to 8p23 are characterized by a higher prevalence of obesity than diabetic individuals with MODY linked to other loci. The nature of this association is unclear at this time. The observation that diabetic members of the 8p23 families have significantly higher body weight than their nondiabetic relatives seems to indicate that obesity is part of the phenotype caused by the disease allele, rather than a predisposing factor. However, these findings must be interpreted with caution because of possible effects of variability in diabetes stage or treatment as confounders of body weight

A



B

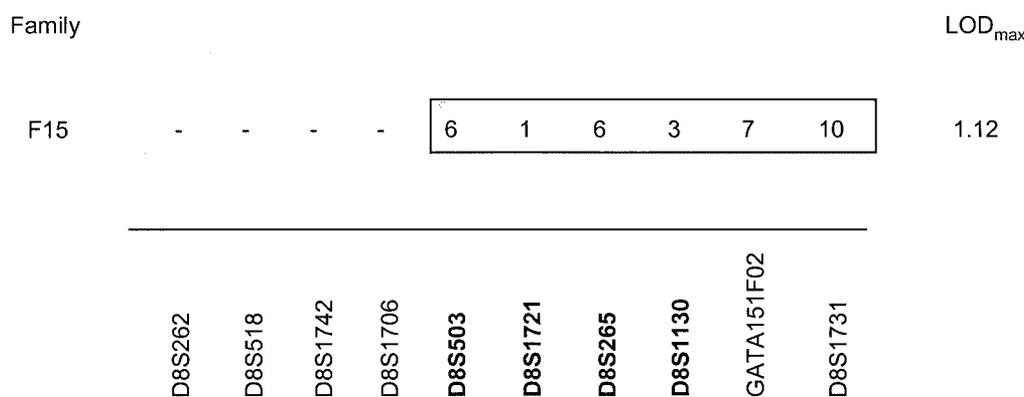


FIG. 2. Haplotype analyses of the three families who showed the highest LOD scores on chromosome 8p23. **A:** Families with the diabetes-linked haplotype carrying the inversion. **B:** Family with the diabetes-linked haplotype not carrying the inversion. Markers placed within the inversion are indicated in boldface. The boxes indicate regions that are shared identical by descent by all affected members within each family. The allele numbering is arbitrary but consistent across families. The minus signs indicate regions that did not segregate with diabetes in all affected family members. The dashed lines indicate the critical interval supported by the haplotype analysis.

measurement. The analysis of nonpenetrant individuals, i.e., those who carry the disease haplotype but do not develop diabetes, might provide useful clues on this issue. Unfortunately, the number of these subjects ($n = 4$) is too small in our families to draw meaningful conclusions.

Other studies have reported linkage of diabetes near our peak at 22 cM from 8pter. In 43 extended Joslin families (247 individuals) with adult-onset type 2 diabetes, a non-parametric LOD score of 3.67 was detected at 7.6 cM (41). In a study of 743 English and Irish sibling pairs with common type 2 diabetes, Wiltshire et al. (42) reported a linkage peak at 42 cM. In a large pedigree of indigenous Australians with type 2 diabetes, linkage was observed at 31.7 cM (43). Also, modest evidence for linkage of type 2 diabetes has been found at 44 cM in multigenerational families of European ancestry (44) and at 15 cM in affected sibpairs from Japan (45). Thus, multiple studies in a variety of populations point to this region. One hypothesis is that the same gene underlies linkage in all of these

studies, with differences in the peak location being due to the occurrence of phenocopies or other factors that affect mapping precision. Allelic heterogeneity or familial factors such as the prevalence of obesity may be responsible for variable age at manifestation of the disease. Alternatively, different diabetes genes may underlie the young- and late-onset loci, their occurrence in the same genomic region being purely coincidental.

The markers that show the greatest evidence of linkage with diabetes in our families (D8S1130 and D8S265) are placed within a 4.7-Mb inversion polymorphism. This polymorphic segment is flanked by two highly homologous, repeated elements that are rich in olfactory receptor genes and that have been implicated in the genesis of other chromosomal rearrangements (35–37). Submicroscopic inversions have been shown to cause heritable disorders (e.g., Hunter's syndrome) by disrupting genes placed at the breakpoints or by displacing regulatory elements of adjacent genes (46,47). However, the relatively high frequency

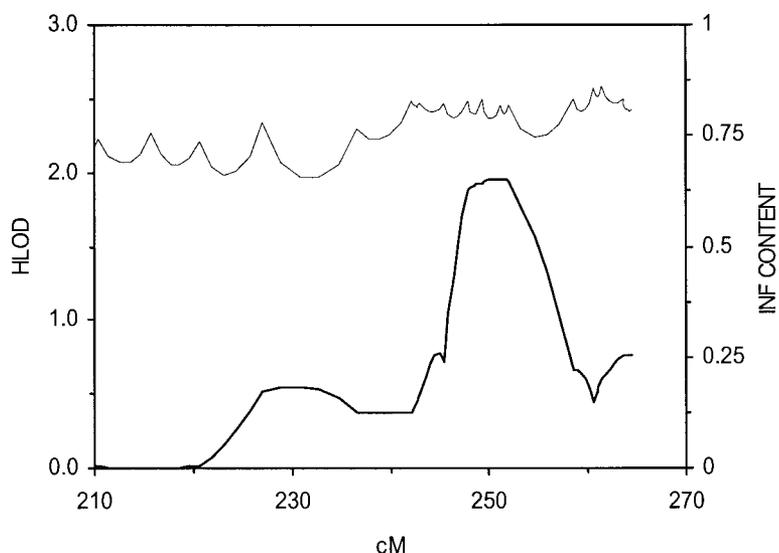


FIG. 3. Multipoint parametric linkage analysis on chromosome 2q37 after typing additional markers. LOD score under the assumption of heterogeneity (HLOD; thick line) and information content (thin line) were calculated by the GENEHUNTER-PLUS program, as described in the text.

of the 8p23 inversion in the general population together with the fact that this variant was found in only two of the three “linked” families examined by FISH argue against a primary role of this polymorphism in the rare form of diabetes segregating in our families, although an ancillary role through an interaction with other variants cannot be excluded at this time.

The presence of the inversion in a substantial proportion of individuals complicates the effort to determine the boundaries of the critical interval as a result of possible differences in the marker order among families. We overcame this obstacle by determining the orientation of the haplotypes segregating with diabetes by means of dual-color FISH. This allowed us to assign a specific marker order to each informative family. With this information, we could narrow the critical region to the interval between D8S1706 and D8S1721 on the “inverted” map. In the latest human genome assembly, which is based on the noninverted orientation, this corresponds to two separate genomic segments: from D8S1706 to the inversion telomeric breakpoint (placed within distal repeat; see Fig. 1A) and from D8S1721 to the centromeric breakpoint (placed within proximal repeat). Together, these two segments span ~2.7 Mb and contain 57 known genes (from RefSeq or other

human mRNAs) plus six others predicted by the National Center for Biotechnology Information’s GenomeScan.

In addition to a potential MODY gene located on 8p23, a smaller linkage peak was observed on chromosome 2q37, corresponding to five families who showed a variable degree of linkage at this location. These five families are distinct from those who are linked at 8p23 and include two Hispanic and one Pacific Islander kindred. The peak is at the same position as *NIDDM1* in Mexican Americans with a later onset of type 2 diabetes (40). Evidence has been produced that *NIDDM1* corresponds to calpain 10, a protease that modulates a variety of cellular pathways by cleaving specific substrates and causing activation or inactivation of protein functions (48). However, in the latest human genome assembly, the calpain 10 gene is placed 500 Kb telomeric to our critical interval (D2S2205 to D2S2253), suggesting that this may not be the gene responsible for linkage at this location in our families.

Five other regions, on 11p15, 1q42, 19q12, 2q24, and 4p13, showed some evidence supporting linkage to MODY. The peak on chromosome 11p15 was mostly due to a single, large pedigree. Recombinants in this family seem to exclude the sulfonylurea receptor 1 (*ABCC8*), a gene placed in this region that has been recently implicated in

TABLE 6

Clinical characteristics of MODYX individuals based on the genetic subgroups

	MODY3	MODYX			P value*
		2q37	8p23	All others	
Families (n)	13	5	6	10	
Affected individuals (M/F)	100 (44/56)	24 (9/15)	44 (15/29)	65 (26/39)	
IGT/gestational diabetes (%)	14.0	12.5	6.8	16.9	0.50
Age at diagnosis (years)	21 ± 10	30 ± 15	31 ± 17	30 ± 17	0.27
Age at examination (years)	39 ± 17	39 ± 15	45 ± 18	47 ± 18	0.18
IBW diabetic individuals (%)	122 ± 22	135 ± 28	141 ± 35‡	123 ± 25	0.004
IBW nondiabetic individuals (%)†	118 ± 23	133 ± 23	118 ± 25	120 ± 22	0.038
Treatment					
Diet only (%)	34.0	25.0	20.4	27.7	
Oral agents (%)	25.0	25.0	25.0	16.9	
Insulin (%)	41.0	50.0	54.6	55.4	0.79
HbA _{1c} (%)	7.0 ± 1.5	8.3 ± 1.5	8.2 ± 1.9	7.6 ± 1.7	0.45

Data are means ± SD. *P value for the comparison across MODYX families. †n = 84 in MODY3, 19 in 2q37, 18 in 8p23, and 67 in MODYX. ‡P = 0.042 for affected vs. nonaffected 8p23 family members.

the cause of an autosomal dominant form of type 2 diabetes (49). We could not find mutations in the insulin gene, which is placed in the critical interval, but the 5' VNTR allele carried by the disease haplotype belongs to class III, a group of alleles that have been associated with increased susceptibility to multifactorial type 2 diabetes (50). One possibility is that the class III allele segregating in this family is a rare variant with an exceptionally high diabetogenic potential, leading to high penetrance and a Mendelian pattern of inheritance. With respect to chromosome 1q42, the location of the strongest evidence of linkage is ~50 cM telomeric to the region where linkage with type 2 diabetes has been detected in families from the U.K., France, and the U.S. (42,44,51,52). Rather, it is located at the same position where linkage with mean glucose has been reported in the Framingham Offspring study (53). The linkage peak on chromosome 19q12 is narrow, spanning <10 cM. This region is placed 15 cM from a linkage peak (maximum LOD score = 1.26) that was described in French pedigrees with a young onset of type 2 diabetes (51) and 27 cM from the insulin receptor gene. The peak on 2q24 corresponds to the location of linkage with diabetes (LOD = 3.9) in a large Australian pedigree (43). The signal on 4p13 is located ~20 Mb from a gene (*WFS1*) responsible for Wolfram syndrome—a disorder characterized by juvenile diabetes, diabetes insipidus, optic atrophy, and deafness (54).

We previously reported linkage of diabetes to chromosome 12q15 in 32 Joslin families who had at least two family members whose type 2 diabetes was diagnosed at age 35 or younger (18). The present genome screen was performed in those families who met a more stringent criterion for a young onset, namely average age at diagnosis in the family equal to 35 years or younger. The four families who were linked to 12q15 in the previous report were not included because their average age at diagnosis was older.

The results of another genome scan of MODYX families from Sweden, France, and the U.K. were published recently (55). Overall, there is little overlap between the results of that study and our linkage peaks. The only common region is on chromosome 6, where we observed an HLOD of 0.88 at 80 cM and Frayling et al. (55) detected an NPL score of 2.12 at 71 cM. No signal was observed at 8p23 in the MODYX families from Europe. This lack of reproducibility is not unexpected given the known genetic heterogeneity of MODYX, with different genes accounting for diabetes in different families. In the presence of heterogeneity, the chances for a disease locus to reach the detection threshold depend on the number and the size of the families who happen to be linked to that locus in that particular study. With relatively small family collections such as these, random differences in the proportion of linked families may have produced dramatic differences in the power to detect specific MODY loci. In addition to this random variability, systematic differences between the two family collections may justify the discrepancies. First, 28% of our families are from minorities, whereas the European study is almost entirely made up of white families. This difference may be especially relevant for the 8p23 and 2q37 loci given that more than half of the families who are linked to these regions were from minorities.

Second, the selection criteria and the resulting phenotypic characteristics differ between family collections. The MODYX families from Europe were selected on the basis of one affected subject whose type 2 diabetes was diagnosed at age ≤25 and the presence of type 2 diabetes in at least two generations. Our families were instead selected on the basis of a within-family average age at diagnosis of 35 years or younger and occurrence of diabetes in three generations or more. On average, our study subjects were diagnosed at a slightly older age (31 vs. 29 years), were heavier (BMI of 28.3 vs. 24.3 kg/m²), and were more frequently treated with insulin (52 vs. 44%, respectively) than the MODYX subjects from Europe. Considering that the families who are linked to 8p23 and 2q37 were characterized by obesity, the lower body weight may be especially important in explaining the absence of linkage at these locations in the European study.

In summary, we have identified a MODY locus on chromosome 8p23 that may account for 30% of MODY cases not caused by known MODY genes. Another MODY gene may be present on chromosome 2q37 and account for some of the remaining families. Positional cloning of these new MODY genes carries with it the possibility of identifying new pathways that may be involved in the pathogenesis of more common forms of type 2 diabetes as well as MODY and provide opportunities to develop new treatments.

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