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A Genetic Map of Chromosome 20q12-q13.1: Multiple Highly Polymorphic Microsatellite and RFLP Markers Linked to the Maturity-Onset Diabetes of the Young (MODY) Locus

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Summary

Multiple highly polymorphic markers have been used to construct a genetic map of the q12-q13.1 region of chromosome 20 and to map the location of the maturity-onset diabetes of the young (MODY) locus. The genetic map encompasses 23 cM and includes 11 loci with PIC values >.50, seven of which have PICs >.70. New dinucleotide repeat polymorphisms associated with the D20S17, PPGB, and ADA loci have been identified and mapped. The dinucleotide repeat polymorphisms have increased the PIC of the ADA locus to .89 and, with an additional RFLP at the D20S17 locus, the PIC of the D20S17 locus to .88. The order of the D20S17 and ADA loci determined genetically (cen–ADA–D20S17–qter) was confirmed by multicolor fluorescence in situ hybridization. The previously unmapped PPGB marker is closely linked to D20S17, with a two-point lod score of 50.53 at $\hat{\theta}$ = .005. These markers and dinucleotide repeat markers associated with the D20S43, D20S46, D20S55, D20S75, and PLC1 loci and RFLPs at the D20S16, D20S17, D20S22, and D20S33 have been used to map the MODY locus on chromosome 20 to a 13-cM (sex averaged) interval encompassing ADA, D20S17, PPGB, D20S16, and D20S75 on the long arm of chromosome 20 and to create a genetic framework for additional genetic and physical mapping studies of the region. With these multiple highly polymorphic loci, any MODY family of appropriate size can be tested for the chromosome 20 linkage.

Introduction

Maturity-onset diabetes of the young (MODY) is a form of non-insulin-dependent diabetes mellitus (NIDDM; type 2 diabetes) which has many clinical characteristics of NIDDM but an early age at onset and an autosomal dominant mode of inheritance (reviewed in Fajans 1990). Recent studies have mapped MODY, as expressed in one large family, the R.-W. family, to the long arm of chromosome 20 (Bell et al. 1991; Bowden et al. 1992*b*; Fajans et al. 1992). Two branches of this large pedigree had documented expression of NIDDM at an early age (i.e., at younger than 25 years old in many members). Two other branches had no documentation of early-onset diabetes, and evaluation of linkage analysis data by using tests for homogeneity strongly suggested that NIDDM in the late-onset branches of the pedigree is not linked to chromosome 20 (Bowden et al. 1992*a*). In this latter study, two other independently ascertained MODY families also showed no evidence of linkage to chromosome 20 markers, and, in addition, two cases of possible nonpenetrance of the MODY phenotype in the R.-W. family were demonstrated (Bowden et al. 1992*a*). Further evidence for heterogeneity in MODY

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was provided by the description of linkage to polymorphisms associated with the glucokinase gene on chromosome 7 in some French (Froguel et al. 1992) and in a British MODY pedigree (Hattersley et al. 1992) and by the subsequent demonstration, by Vionnet et al. (1992), of a nonsense mutation in the glucokinase gene of one of the families.

Because of the genetic heterogeneity of MODY and the possibility of nonpenetrance in chromosome 20–linked families, it is important to have highly informative polymorphic markers to test additional MODY families for linkage to chromosome 20 and to evaluate heterogeneity within families. The RFLP at locus D20S16 is tightly linked to MODY (Bowden et al. 1992*a*, 1992*b*) and is very informative (Donis-Keller et al. 1987; Schumm et al. 1988), but D20S16 reveals a complex pattern of allelic fragments (Schumm et al. 1988; Bowden et al. 1992*a*) which make it difficult to follow segregation patterns. For these reasons, we have searched for additional highly informative genetic markers. These markers, in conjunction with several newly described microsatellite polymorphisms (Hazan et al. 1992; Hudson et al. 1992), have been used to construct a refined genetic map of 20q12-q13.1, which should help precisely define the location of the MODY gene and act as a framework for positional cloning of the MODY gene.

Material and Methods

Locus Expansion and Identification of Additional Polymorphisms at the D20S17, ADA, and PPGB Loci

Locus expansion was carried out in an effort to increase the informativeness of the ADA locus, the poorly informative D20S17 locus, and the nonpolymorphic PPGB locus. In an effort to find new polymorphic markers for ADA, PPGB, and D20S17, genomic clones were isolated from a cosmid library by using a previously described efficient screening method (Bowden et al. 1988) with the ADA cDNA clone ADAN16 (Daddona et al. 1984), the PPGB cDNA clone HuPP (Galjart et al. 1988), or the CRI-L127 (D20S17; Donis-Keller et al. 1987) genomic DNA insert as probe. Multiple overlapping cosmids homologous to ADA, PPGB, or D20S17 were isolated and used as radiolabeled probes for screening random human genomic DNAs for RFLPs according to a method described elsewhere (Donis-Keller et al. 1987; Schumm et al. 1988; Bowden et al. 1989*b*). This screening resulted in one informative *Taq*I RFLP, designated "L127/S89," at D20S17 (tables 1 and 2). The cosmids were also screened for dinucleotide repeat sequences according to a method described by Rothschild et al. (1992). Restriction-enzyme fragments containing dinucleotide repeat sequences were subcloned into M13 and sequenced, and oligonucleotides flanking the dinucleotide repeat sequences were designed for PCR amplification essentially according to the method described by Rothschild et al. (1992). Primers and other characteristics of the D20S17, PPGB, and ADA dinucleotide repeats are summarized in table 1. The sequences of the clones from which primers were identified were as follows: D20S17 primers L127pr1/pr2, $(AC)_3AT(AC)_{18}$; D20S17 primers L127pr7/pr8, $(CA)_6TG(CA)_4CG(CA)_{14}$; PPBG primers PPGBpr1/pr2, $(CA)_{18}$; and ADA8 primers, $(CA)_{21}$.

PCR amplification with D20S43, D20S46, D20S55, PLC1, and both D20S17 sets of primers employed incorporation of α^{35}S-dATP and was carried out according to a method described by Rothschild et al. (1992), by using an annealing temperature of 58°C for all primer pairs except D20S55 (annealing temperature 55°C). These conditions did not work for PCR amplification with the ADA8 primers, presumably because of the presence of A-rich regions flanking the dinucleotide repeat. PCR with the ADA8 primers was carried out in a 25-μl volume containing 250 ng of genomic DNA, 5–10 ng of primer 1 end-labeled with γ^{35}P-ATP, 50 ng of unlabeled primer 1, 5 ng of primer 2 (which has homology to the *Alu* repetitive sequence), 200 μM of each dNTP, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% BSA, 1 mM spermidine, and 1 unit of *Taq* polymerase (Perkin Elmer Cetus). The ADA8 reactions were processed through 10 temperature cycles consisting of 1 min at 94°C, 1 min at 59°C, and 1 min at 72°C, followed by 15 temperature cycles consisting of 1 min at 91°C, 1 min at 59°C, and 1 min at 72°C, with the last elongation step lengthened to 6 min. PCR amplification of the ADA AluVpA polymorphism (primers OL3 and OL4) was carried out according to a method described by Economou et al. (1990), and genotyping with D20S75 was carried out with primers and conditions described by Hudson et al. (1992). Products of all PCR reactions were analyzed on standard sequencing gels (6%) according to a method described by Weber and May (1989).

Analysis of the inheritance of RFLP loci was carried out using methods for purification and radiolabeling of probe DNAs, restriction-enzyme digests of human DNAs, agarose gel electrophoresis, and Southern blot transfer of the human DNAs, hybridization, and auto-

Table 1

New Dinucleotide Repeat Polymorphisms and RFLPs Associated with the D20S17, PPGB, and ADA Loci

Locus	Oligonucleotides	No. of Alleles	Allele Size (bp)	Allele Frequency (%)
D20S17	L127PR1 AAGACTGAGCCCAAGTCATTTGC L127PR2 CAGGATCTCTCTGCAAGGACTC	6	196	1.3
			198	51.3
			200	28.9
			202	14.1
			204	3.3
			206	1.3
D20S17	L127PR7 GGCTAAGTATGCAGCAGTTAG L127PR8 GTACTTTCTCTTTGCAGACCTTG	6	197	3.8
			199	35.2
			201	10.9
			203	29.5
			205	18.0
			207	2.6
PPGB	PPGBPR1 GATGAGAAGACCCTTCAACC PPGBPR2 GATTGGGCCTTATGAGCAGG	8	209	.9
			207	.9
			205	3.1
			203	24.9
			201	46.3
			199	15.5
			197	7.2
			191	1.2
ADA	ADA8PR1 CCAGCCTGGCTGAAAGAGTG ADA8PR2 CAATGTCCTCCAGGTTCAACC	7	170	3.5
			172	14.2
			174	7.1
			176	32.1
			178	17.9
			180	17.9
			182	7.1

Locus	Probe	No. of Alleles	Allele Size (kb)	Allele Frequency	Constant Fragments (kb)
D20S17	L127/S89	2	8.5	.46	6.0, 5.6, 2.1
			4.5, 2.9	.54	

radiography as described by Donis-Keller et al. (1987) and Schumm et al. (1988).

Polymorphic Markers

Highly polymorphic microsatellite and conventional RFLPs used in this study are listed, in table 2, by locus designation, polymorphism type, PIC, and reference. For the D20S17 and ADA loci, multiple allelic systems associated with the loci are listed individually and with the cumulative PIC of the systems. PIC values were calculated using the LINKAGE utility program PIC v. 1.3 (Ott 1991). In addition to genotypic data collected with these markers in the MODY family, additional data previously collected with other RFLP systems—D20S5, D20S6, D20S18, D20S4, and D20S15 (Donis-Keller et al. 1987; Bowden et al. 1992*a*, 1992*b*)—were used for map construction and multipoint linkage analysis.

Construction of a Genetic Map of the Chromosome 20q12-13.2 Region

A genetic map of chromosome 20 was constructed using data from the Centre d'Etude du Polymorphisme Humain (CEPH) data base, version 5, as well as by using genotypic data collected on the CEPH families with the dinucleotide repeat polymorphisms associated with the L127pr1/pr2, PPGB, and D20S75 primers (tables 1 and 2), the PLC1 gene locus (Rothschild

Table 2

Highly Polymorphic Markers

Locus	Probe Name	Polymorphism Type	PIC	Reference
PLC1	plcpcr1,2	Dinucleotide repeat	.72	Rothschild et al. 1992
D20S46	IP20M77	Dinucleotide repeat	.81	Hazan et al. 1992
D20S55	IP20M3	Dinucleotide repeat	.76	Hazan et al. 1992
D20S43	IP20M66	Dinucleotide repeat	.56	Hazan et al. 1992
ADA	. . .	AluVpA	.76	Economou et al. 1990
	ADApcr8	Dinucleotide repeat	.77	Present paper
		Cumulative	.89	
D20S17	CRI-L127	RFLP (*MspI*)	.31	Donis-Keller et al. 1987
	L127/S89	RFLP (*TaqI*)	.35	Present paper
	L127-CA-P1,P2	Dinucleotide repeat	.53	Present paper
	L127-CA-P7,P8	Dinucleotide repeat	.70	Present paper
		Cumulative	.88	
PPGB	PPGB-CA-P1,P2	Dinucleotide repeat	.65	Present paper
D20S22	pFMS22-1.4	RFLP (*MspI*)	.54	Stolz et al. 1991
D20S16	CRI-LI214	RFLP (*BglII*)	.98	Donis-Keller et al. 1987
D20S75	MIT-M127	Dinucleotide repeat	.84	Hudson et al. 1992
D20S33	CEB2	RFLP (*PstI*)	.57	Vergnaud et al. 1991

et al. 1992), and the AluVpA polymorphism (Economou et al. 1990; data provided by S. E. Antonarakis, Johns Hopkins University). Genotyping with the D20S17, PPGB, D20S75, and PLC1 polymorphisms was carried out on the standard panel of 40 CEPH families. D20S16 was genotyped on an additional 19 of these 40 families not previously genotyped. These data were merged with genotypic data collected on the R.-W. family (see below), which added an additional 76 potentially informative meioses. Map construction was carried out as described in detail by Bowden et al. (1989*a*), by using the CRI-MAP linkage analysis program, version 2.4. The initial map was constructed at 1,000:1 odds by using the BUILD option of CRI-MAP. The tentative order of markers from BUILD was evaluated further with the FLIPS option of CRI-MAP, which permutes sets of adjacent loci within the map and calculates the corresponding likelihoods for these orders. FLIPS was run permuting orders of three adjacent loci to search for map orders with likelihoods higher than that of the initial map. Map distances are calculated using the Kosambi mapping function with this program. The CHROMPICS option of CRI-MAP was used to rigorously check all data, and apparent isolated double crossovers were tested by repeating the genotypings. Log_{10} likelihoods for fixed orders of markers were calculated using the FIXED option of CRI-MAP. *P* values for the χ^2 test for heterogeneity (Ott 1985) were calculated using the LINKAGE utility program CHIPROB (Ott 1991).

Fluorescence In Situ Hybridization (FISH)

Peripheral blood leukocytes from chromosomally normal donors were cultured using standard laboratory techniques. Chromosome preparations were obtained using both a standard colcemid technique (0.8 ml of 10 μg/ml) and a modified ethidium bromide procedure (Ikeuchi 1984). Slide preparations were stored at −20°C.

Cosmid probes for ADA and D20S17, as well as the bacteriophage lambda genomic clone for D20S16 (CRI-L1214), were labeled by nick-translation with either digoxigenin-11-dUTP (Boehringer Mannheim, Germany) or biotin-14-dATP (GIBCO-BRL, Gaithersburg, MD) by using the BioNick labeling system (GIBCO-BRL, Gaithersburg, MD). FISH was carried out with the probes singly or in combination with each other and also simultaneously with a chromosome 20 centromere-specific probe D20Z1 (ONCOR, Gaithersburg, MD) by using modifications of the manufacturer's Blockit (ONCOR, Gaithersburg, MD) protocols. The probes were prehybridized together at 37°C in hybrisol VI for 2–3 h and then were applied to the denatured slides.

Prior to hybridization, slides were treated with RNase A (100 μg/ml in 2 × SSC) at 37°C for 1 h, rinsed in 2 × SSC, dehydrated in a series of ethanol washes, and air-dried. The slides were then denatured in 70% formamide/SSC, rinsed in 2 × SSC, ethanol dehydrated, and air-dried. The hybridization mixture

was applied to prewarmed slides, sealed with a coverslip, and incubated overnight at 37°C in a humidified chamber. Slides were washed at 43°C with constant agitation for 20 min in 50% formamide/SSC, 2 × SSC and were placed in 1 × PBD (phosphate-buffered detergent) at room temperature.

Detection was performed using a modified protocol of Oncor (ONCOR, Gaithersburg, MD). Equal amounts of fluorescein-labeled avidin (FITC) and anti-DIG-rhodamine were applied to each slide for 20 min in a humidified chamber at 37°C. Slides were washed in 1 × PBD. Amplification was performed according to the protocol of the Oncor amplification kit. Chromosomes were counterstained in DAPI/Antifade solution, were covered with a glass coverslip, and then were examined with a Zeiss Axiophot microscope equipped with epifluoresence filters for DAPI, FITC, and XRITC. Images were captured and stored on TOMS Image Analysis System (TOMS, Atlanta), and prints were from a SONY color printer. Neither image enhancement nor color assignment was performed with this system.

Genetic Mapping of the MODY Locus

Linkage of MODY to chromosome 20 polymorphic markers was evaluated using the LINKAGE analysis program (version 4.8) (Lathrop et al. 1984), which can incorporate estimates of age-related penetrance. The MLINK and ILINK options were used for two-point analysis, and LINKMAP was used for multipoint analysis. Genotypic data were collected from the two branches (II-2 and II-5) of the R.-W. family which have documented early onset of diabetes (MODY) and in which diabetes is clearly linked to chromosome 20 (Bell et al. 1991; Bowden et al. 1992*a*). We now have DNA samples from 88 members of these two branches. Age-related penetrance was incorporated into the analysis by using a stepwise age-at-onset estimate assuming complete penetrance and five liability classes as described in detail elsewhere (Bowden et al. 1992*a*). LINKMAP analysis was carried out using a sliding group of three fixed loci, inserting the test (disease) locus. Lod scores are reported for calculations in interval "1" (according to the terminology of Ott 1991). With the highly polymorphic loci and the large pedigree, the LINKMAP calculations could only be carried out by splitting the pedigree into two separate families and, in addition, recoding haplotype data to reduce the number of alleles. Calculations were carried out on a VaxStation 3100 M48 computer.

Results

Development of Highly Polymorphic Loci in the Chromosome 20q12-q13.1 Region

In order to refine the genetic location of the MODY locus and to improve our ability to evaluate other MODY families for linkage to chromosome 20, we have developed additional polymorphic markers in the 20q12-q13.1 region. The D20S16 locus evaluated in earlier studies (Bowden et al. 1992*a*, 1992*b*) is uniquely informative, with a PIC of .98 (Donis-Keller et al. 1987), and is informative in all matings in the branches of the R.-W. family which were analyzed in the present study. The complex pattern of allelic fragments revealed at D20S16, however, makes assignment of genotypes difficult in many families. Other markers were not as informative (Bowden et al. 1992*a*, 1992*b*). The locus D20S17 (CRI-L127), for example, was initially very poorly informative in the MODY family (Bowden et al. 1992*b*). Our ability to rapidly and efficiently isolate cosmid clones (Bowden et al. 1988), combined with the high frequency of dinucleotide repeat sequences in genomic fragments of 25–50 kb (Weber and May 1989), enabled us to find new informative polymorphisms for the PLC1 gene (Rothschild et al. 1992), the previously unmapped PPGB gene, and the D20S17 and ADA loci, as outlined in tables 1 and 2.

Several dinucleotide repeat sequences were detected in the cosmid clones containing D20S17, PPGB, and ADA. Two different polymorphic dinucleotide repeat sequences were characterized from cosmids containing D20S17, and one each was characterized from cosmids homologous to the PPGB and ADA loci. The oligonucleotide primers for PCR amplification of these sequences, as well as allele sizes and frequencies in the CEPH and MODY families, are shown in table 1. In addition, a simple two-allele *Taq*I RFLP was revealed with a 10-kb fragment derived from a cosmid (designated L127/S89) from D20S17 (table 1).

As can be seen in table 2, addition of new markers for ADA and especially for the D20S17 locus significantly increased the PIC of these loci. When all four of the polymorphisms were used to construct haplotypes, the cumulative PIC for D20S17 rose from .35 for the original CRI-L127 to .88. This great increase in informativeness was also reflected in seeing 16 different haplotypes in the R.-W. family. The corresponding increase in the PIC for the ADA locus was not as great, since the original ADA AluVpA polymorphism has a high PIC, but the additional dinucleotide repeat poly-

morphism was very useful in mapping MODY (see below). There were 22 different haplotypes in the R.-W. family when data from the ADA AluVpA and ADApcr8 markers were combined. The dinucleotide repeat sequence flanked by the ADApcr8 primers has a neighboring associated A-rich sequence, which we presume made initial attempts at PCR amplification unsuccessful. This locus was successfully amplified with the conditions outlined in Material and Methods.

Genetic Map of Chromosome 20q12-q13.1

Figure 1*A* shows the sex-averaged genetic map constructed using odds of 1,000:1. PLC1;q12-q13.1 (Bristol et al. 1988) and D20S33;q13.1 (Vergnaud et al. 1991) have been used as markers for the boundaries of the q12-q13.1 region, since they are, respectively, the most proximal and most distal genetically mapped loci which have been physically assigned to these bands. ADA (Jhanwar et al. 1987), D20S17 (Barr et al. 1991; Schnittger and Hansmann 1991), D20S16 (Rao et al. 1992), PPGB (Wiegant et al. 1991), and D20S22 (Stolz et al. 1991) have also been localized to the chromosome 20q12-q13.1 region. Several additional markers, which have been physically localized to other regions of chromosome 20 and have also been genetically mapped, were also included. These markers are D20S5;p12 and D20S6;p12 (Goodfellow et al. 1987), D20S18;p11.2 (Schnittger and Hansmann 1991), D20S4;q13.2 (Goodfellow et al. 1987), and D20S15;q13.2 (Collaborative Research technical literature). Only one of the markers in the q12-q13.1 region, D20S22, could not be uniquely placed. Analysis with the CHROMPICS option of the CRI-MAP programs reveals three apparent recombinants between D20S22 and D20S16. Two of these recombinants are in the MODY family and, on the basis of the inheritance of flanking markers, favor the order cen–D20S22–D20S16–qter. One recombinant, in CEPH kindred 1347, suggests the order cen–D20S16–D20S22–qter. When the FIXED option of CRI-MAP is used, the latter order is favored, but only with odds of 8.5:1. At this time we cannot place D20S22 without ambiguity. The PPGB polymorphism is closely linked to the D20S17 locus in two-point linkage analysis (lod = 50.53 at $\hat{\theta}$ = .005) but was uniquely placed relative to D20S17, on the basis of a single recombination event (see Discussion). Local support for the order of markers within the q12-q13.1 region was at least 1,000:1 in the final map, except for D20S22. This was determined using the FLIPS option of CRI-MAP. When PLC1 and D20S33 are taken as the boundaries, the q12-q13.1 region encompasses 23 cM and includes 11 loci with PICs >.50, 7 of which have PICs >.70.

Figure 1*B* shows the sex-specific maps generated with CRI-MAP. The sex-specific distances for the female and male maps in 20q12-q13.1 (markers PLC1 through D20S33) are 33.1 and 15.3 cM, respectively. This difference was significant when the χ^2 test for heterogeneity (Ott 1985) was applied: χ^2 = 34.3 (9 df). The corresponding $P \leq .000079$ indicates that this difference is statistically significant and consistent with other previously published evaluations of sex-specific recombination on chromosome 20 (e.g., see Donis-Keller et al. 1987; Barr et al. 1991). In contrast, only one pair of adjacent loci, D20S43 and ADA, show significant sex-specific differences in the recombination fraction (P = .0005). The distances between adjacent loci, however, are all small (<5 cM).

Physical Mapping of the D20S17 and ADA Loci by FISH

The order of the ADA and D20S17 loci in the maps shown in figure 1 differs both from the order deduced from a subset of the genotypic data (Bowden et al. 1992*a*; Rothschild et al. 1992) used in the present study and also from the order calculated by Hazan et al. (1991) and Barr et al. (1991). With the present data set, the order cen–ADA–D20S17–qter is favored by odds of $>10^{10}$:1 over the order cen–D20S17–ADA–qter. In order to confirm this result, we have physically mapped the loci by using FISH. Results of this analysis are shown in figure 2. Each cosmid probe was mapped singly to the long arm of chromosome 20 by using the biotin-labeled D20Z1 chromosome 20 centromere-specific probe as the chromosome 20 marker. The probes were ordered by alternating in combination each probe (ADA, D20S17, and also including D20S16) labeled with either biotin or digoxigenin and in combination with D20Z1. At least 20 metaphases and interphase cells from each experiment were analyzed. The top panel of figure 2 shows in a metaphase spread the following order: D20Z1–ADA–D20S17. The ordering of D20S17 and D20S16 together in a metaphase spread was not possible, because of the close proximity of the probes—and, hence, overlapping signals. However, in prometaphase cells the order was shown to be D20Z1–D20S17–D20S16 (fig. 2, *bottom*). Thus the order of the probes is D20Z1–ADA–D20S17–D20S16, consistent with the results of genetic analysis.

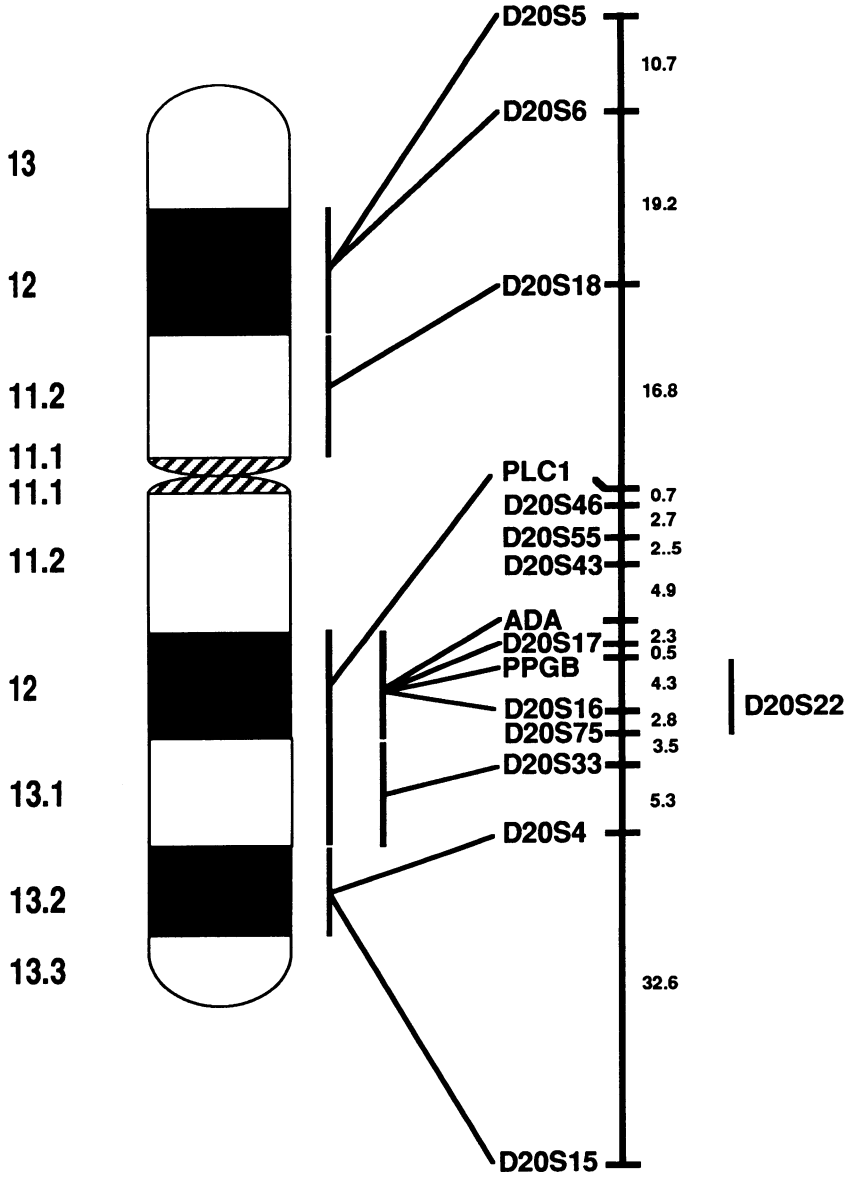
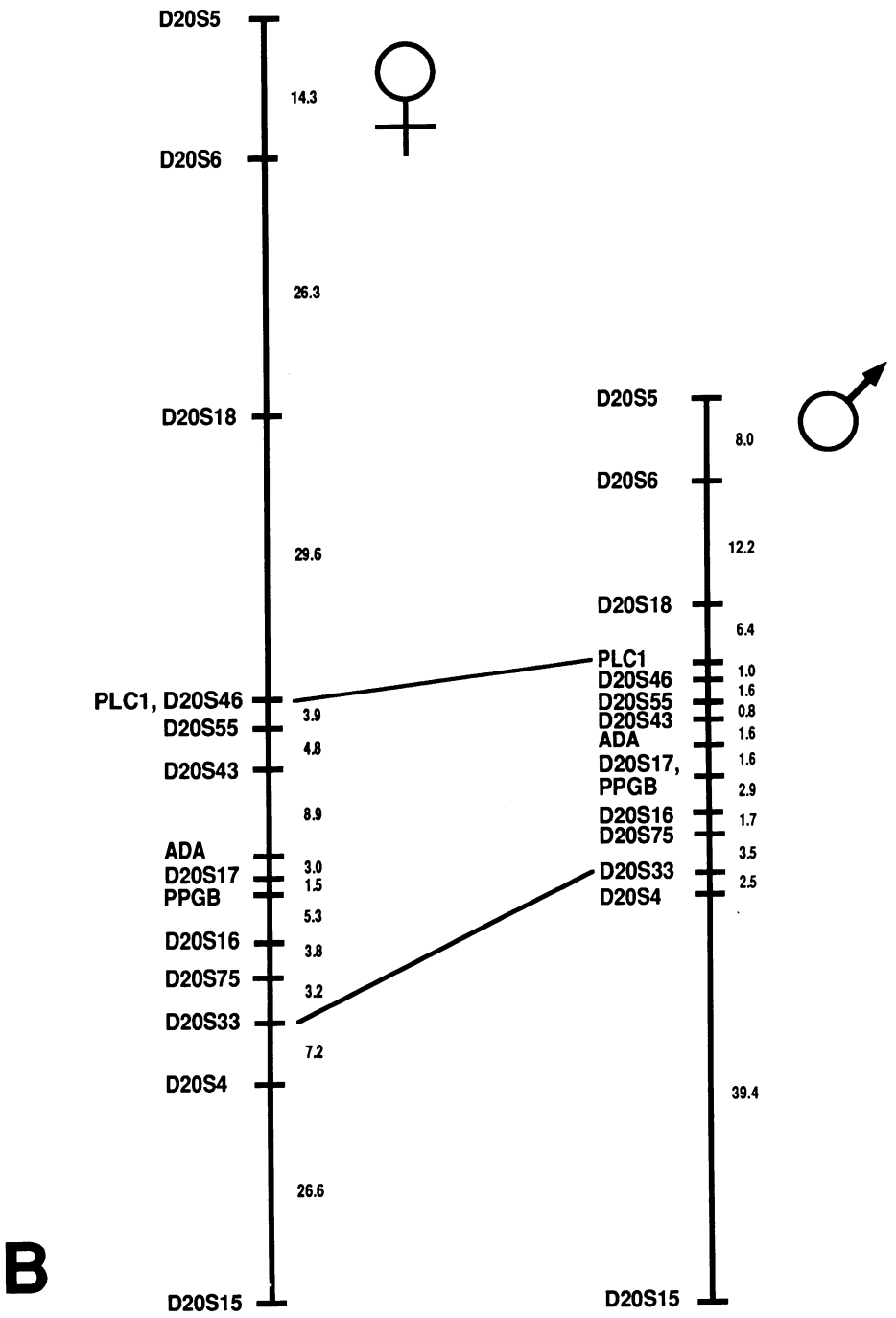


Figure 1 Genetic maps of chromosome 20q12-q13. *A*, Sex-averaged genetic map correlated to the cytogenetic map of the metaphase chromosome 20. *B*, Sex-specific genetic maps of the region. The highest likelihood order of markers was the same whether data for the markers shown in the figures alone were used to construct the map or whether additional data from other markers in the CEPH version 5 data base were also included in the map construction. Lines drawn between PLC1 and D20S33 on the female and male maps are the approximate boundaries of the 20q12-q13.1 region.

Mapping of MODY by 20q12-q13.1 Markers

The highly polymorphic loci in 20q12-q13.1 have been genotyped on the early-onset branches (descendants of II-2 and II-5; Bowden et al. 1992*a*, 1992*b*) of the R.-W. MODY family, and two-point and multipoint linkage analyses have been carried out on the data. Table 3 includes lod scores from two-point linkage, including the maximum lod score and corresponding $\hat{\theta}$ calculated for the early-onset branches, and the maximum lod scores calculated when the two early-onset branches of the family are treated as two different families. The highest lod score is >13.3 at $\hat{\theta} = 0$ with D20S16, which is perfectly informative.



When the branches are analyzed separately, the lod score with D20S16 falls to 12.63 ($\hat{\theta} = 0$), because of loss of phase information when the pedigree is split. The increase in PIC of the D20S17 locus is reflected in the dramatic increase in lod score, from 0.90 for the original *Msp*I RFLP to >12 for the haplotyped system.

The value of multiple highly polymorphic markers, particularly in the analysis of a single large family, can also readily be seen in table 3. If the progenitors of a

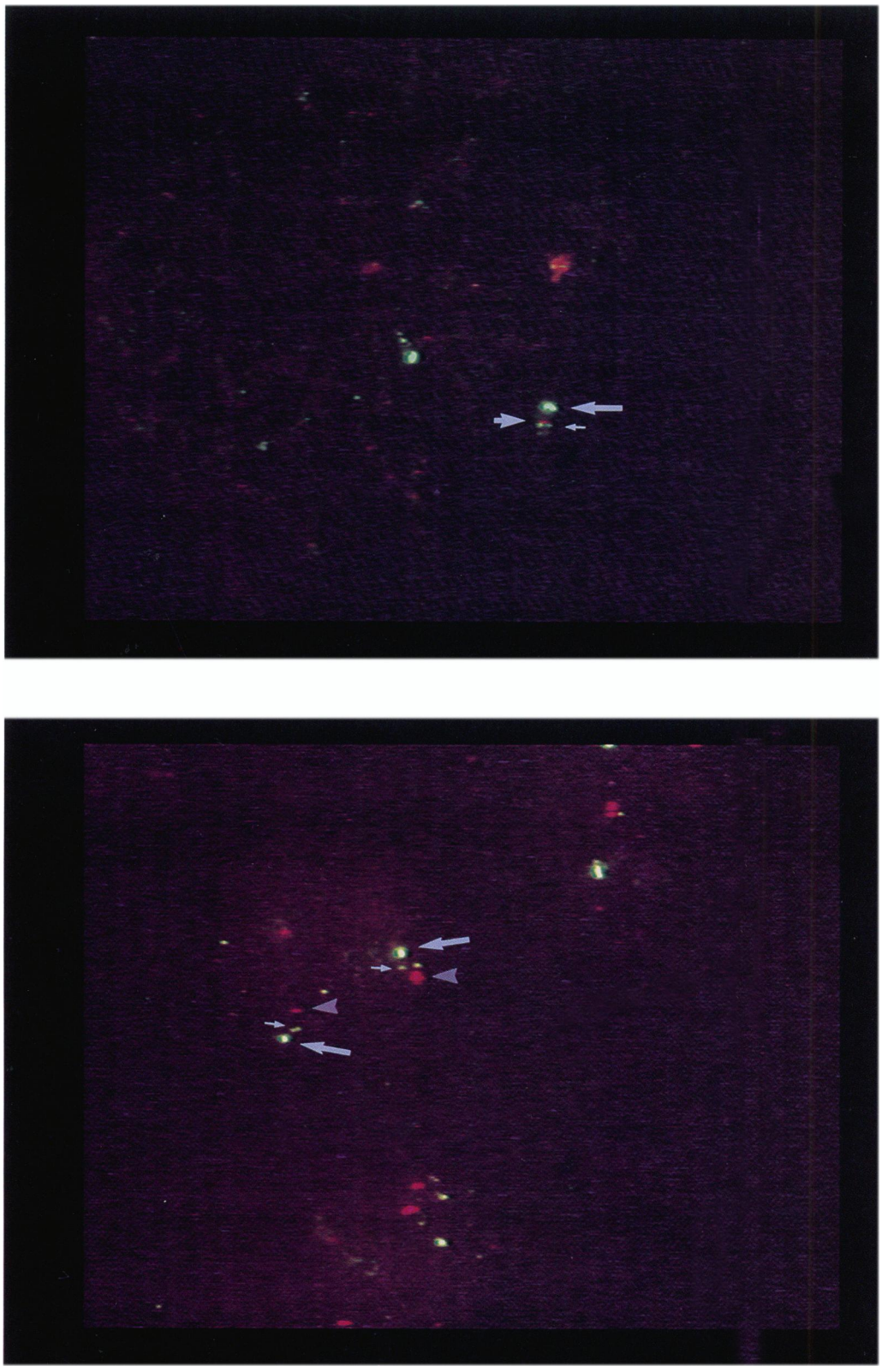


Figure 2 FISH mapping of D20Z1, ADA, D20S17, and D20S16. *Top,* Metaphase spread with the chromosomes showing lightly (reduced suppression). Localization of D20Z1 (yellow; long large arrow), ADA (red; medium-length arrow), and D20S17 (yellow; small arrow) is shown. *Bottom,* Prometaphase cell showing localization of D20Z1 (yellow; large arrow), D20S17 (yellow; small arrow), and D20S16 (red; arrowhead).

Table 3

Two-point Linkage Analysis with Polymorphic Loci from 20q12-q13.1 in the R.-W. Family

Locus	Lod Score at θ =										II-2 + II-5		Maximum Lod Score	
	0	.05	.10	.15	.20	.25	.30	.35	.40	.45	Maximum Lod Score	θ̂	II-2	II-5
PLC1[a]	.23	4.28	4.47	4.25	3.85	3.32	2.70	2.01	1.29	.58	4.47	.09	1.54	2.78
D20S46	8.24	7.60	6.92	6.18	5.39	4.55	3.65	2.70	1.70	.72	8.23	.00	2.28	5.45
D20S55	9.02	9.83	9.27	8.46	7.51	6.45	5.27	3.97	2.57	1.11	9.92	.03	3.21	6.25
D20S43	9.75	10.32	9.63	8.75	7.75	6.65	5.46	4.16	2.76	1.26	10.51	.02	4.29	5.50
ADA:														
AluVpA	8.42	7.77	7.08	6.35	5.56	4.73	3.84	2.89	1.89	.86	8.41	.00	2.08	5.95
ADApcr8	12.97	11.99	10.94	9.82	8.62	7.36	6.00	4.56	3.01	1.38	12.95	.00	4.46	7.80
Haplotype	13.18	12.19	11.12	9.98	8.76	7.47	6.09	4.61	3.04	1.38	13.17	.00	4.46	8.12
D20S17:														
CRI-L127	.90	.82	.74	.64	.55	.45	.34	.25	.15	.07	.90	.00	.61	−.02
L127/S89	2.87	2.60	2.32	2.03	1.73	1.42	1.10	.78	.47	.18	2.87	.00	−.24	3.14
L127-CA-P1,P2	5.98	5.38	4.75	4.08	3.40	2.73	2.09	1.50	.96	.46	5.98	.00	2.13	3.41
L127-CA-P7,P8	9.93	9.16	8.34	7.46	6.54	5.55	4.50	3.38	2.18	.98	9.93	.00	2.28	7.35
Haplotype	12.17	11.23	10.21	9.13	7.98	6.76	5.46	4.09	2.62	1.13	12.17	.00	4.25	7.33
PPGB	5.52	5.00	4.46	3.91	3.34	2.77	2.20	1.63	1.08	.53	5.02	.00	1.88	4.14
D20S22	7.50	6.88	6.21	5.51	4.77	4.00	3.19	2.34	1.46	.61	7.50	.00	4.09	2.91
D20S16	13.32	12.31	11.23	10.08	8.86	7.56	6.17	4.69	3.10	1.43	13.31	.00	4.46	8.16
D20S75	12.60	11.64	10.59	9.47	8.28	7.01	5.66	4.22	2.68	1.10	12.60	.00	4.46	7.79
D20S33	.09	.17	.21	.22	.21	.17	.13	.08	.04	.01	.21	.14	.25	.02

[a] Data are from Rothschild et al. (1992).

large family are homozygous, this lack of informativeness is carried through subsequent generations. An example is seen by comparing the PLC1 and D20S46 loci, which are only 0.7 cM apart and have comparable PIC values (table 2); yet the two markers have markedly different lod scores with the MODY family. This reflects the fact that PLC1 was homozygous in many individuals in the II-2 branch, while D20S46 was highly informative. A similar situation occurred with the ADA locus, where the two allelic systems have virtually the same PIC, yet the original ADA AluVpA polymorphism was only poorly informative in the II-2 branch of the family, while the ADApcr8 polymorphism was fully informative (compare with D20S16). Finally, the D20S33 locus, which is reasonably informative in the CEPH families, is very poorly informative in the MODY family.

Results of the multipoint linkage analysis with MODY are shown in figure 3. The highest likelihood placement of MODY (95% confidence interval) is in a 13-cM (sex-averaged) interval which includes ADA, D20S17, PPGB, D20S16, D20S75, and possibly also D20S22. The maximum lod score is 12.63 at D20S16, reflecting the results with this highly polymorphic locus in two-point linkage analysis. This gives us the unusual result of having a higher lod score with two-point analysis than with multipoint analysis. This is an artifact due to inability to carry out multipoint analysis with this large family and highly polymorphic markers, except by splitting the family to carry out the analysis (see Material and Methods).

Discussion

Several new polymorphic microsatellite sequences and one RFLP have been identified in 20q12-q13.1. Several additional microsatellite polymorphisms which map to this region have also been recently described (Hazan et al. 1992). Mapping data with these markers has been combined with previously described MODY-linked markers D20S16 (Bowden et al. 1992*a*, 1992*b*), ADA (Bell et al. 1991; Bowden et al. 1992*a*), and PLC1 (Rothschild et al. 1992) to construct a genetic map of 20q12-q13.1. The large number of highly polymorphic markers virtually assures that some will be informative in any new MODY families tested for linkage to chromosome 20. In addition, genetic heterogeneity in MODY can be accurately evaluated with

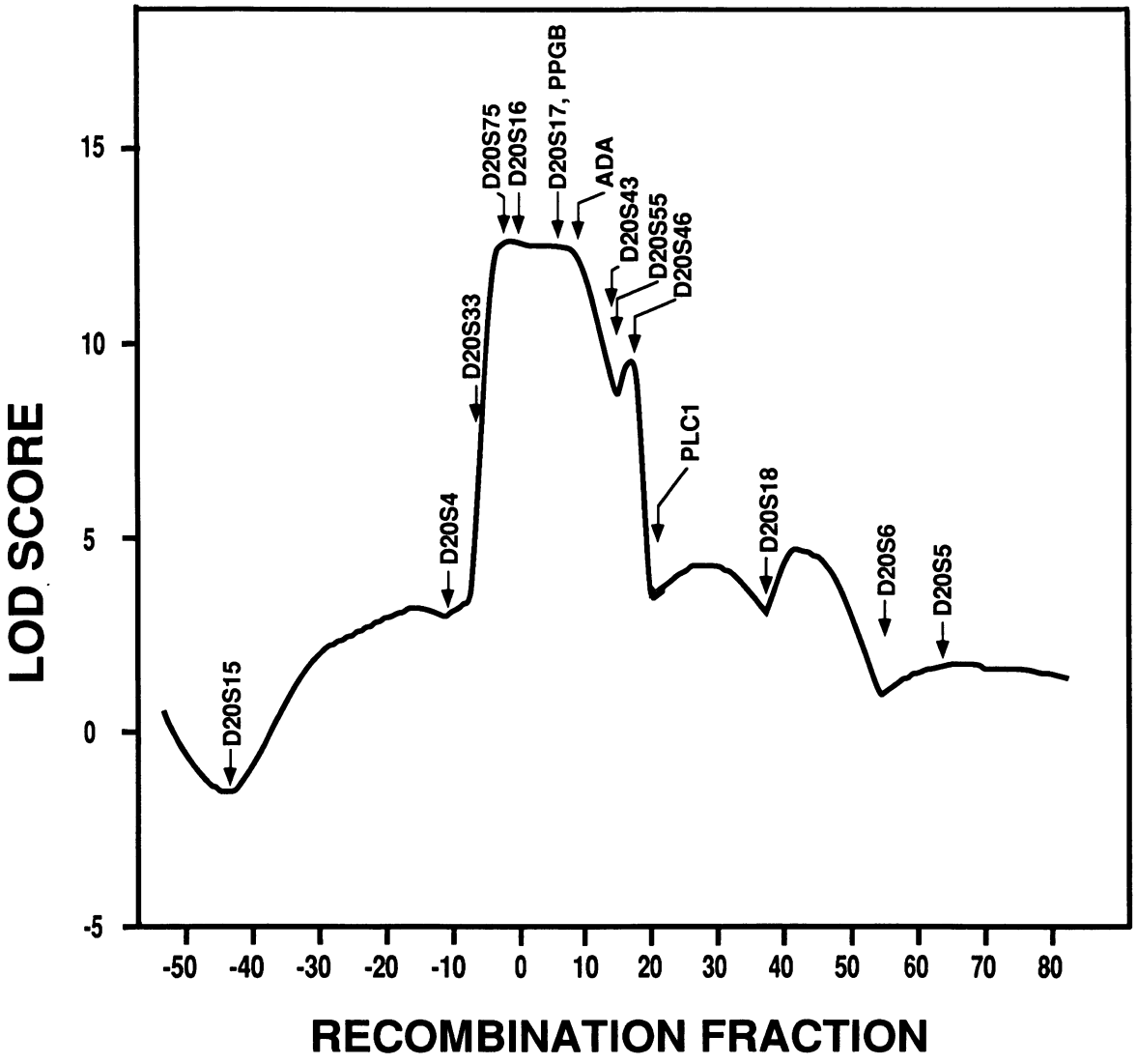


Figure 3 Multipoint linkage analysis of MODY by chromosome 20 polymorphic loci. Lod scores were calculated by placing the MODY locus in each interval within the map of chromosome 20 loci. The arbitrary origin was chosen to be at D20S16, and distances from this locus are shown. The order of polymorphic loci was determined as described in Material and Methods. Calculations are based on sex-averaged analysis assuming autosomal dominant mode of inheritance. The distances between loci are shown in fig. 1*A*.

these multiple highly polymorphic markers. Also, the identification of a polymorphism associated with the PPGB locus may be helpful in the study of inherited galactosialidosis (Andria et al. 1981).

These markers have already proved useful in the evaluation of one family in the II-2 branch of the R.-W. pedigree. One diabetic individual in this branch (III-2, a daughter of II-2; Bowden et al. 1992*b*) was apparently the result of a recombination between MODY and D20S16 (Bowden et al. 1992*a*). This individual, however, was diagnosed as diabetic when she was 48 years old, leading to the possibility that she is not diabetic because of the chromosome 20–linked MODY but because of inheritance of some other diabetogenic gene. This is possible, since diabetes unlinked to chromosome 20 is also segregating in the R.-W. family (Bell et al. 1991; Bowden et al. 1992*a*). Through genotyping several additional individuals from this family with the multiple highly polymorphic markers described here, it can be concluded that individual III-2 has not in fact inherited chromosome 20–linked MODY. MODY inheritance in III-2 could only

be explained if multiple cases of nonpenetrance and recombination events had all occurred in the immediate family. The odds of this are quite low (on the order of 10,000:1; data not shown).

We have been frustrated in our desire to more precisely define the location of the MODY locus. Even though mapping in the CEPH and MODY families gives us a sex-averaged distance of 9.9 cM between D20S75 and ADA (fig. 1*A*), there are no apparent crossovers between the highly informative markers ADA, D20S17, PPGB, D20S22, D20S16, and D20S75 and MODY in 67 potentially informative meioses from the MODY family. This is possibly a reflection of sex-specific recombination demonstrated in the q12-q13.1 region (see Results and fig. 1*B*), since 47 of these meioses are male meioses. Though the distances between ADA and D20S75 are 13.8 cM for the female map and 6.2 cM for the male map, this difference is not significant ($P = .10$, $\chi^2 = 7.69$, df = 4) with the present genotypic data. With collection of mapping data from additional families, the sex-specific difference in recombination may be shown to be significant in this short region.

Our most recent effort to better define the position of MODY (Rothschild et al. 1992) placed the locus in an 11-cM interval around D20S16. The genotyping of six new loci (D20S46, D20S55, D20S43, PPGB, D20S22, D20S75, and D20S33) and additional polymorphisms at ADA and D20S17 have led to the reassignment of the order of D20S17 and ADA. Previously, PLC1 was the closest centromere-proximal marker separated by recombination from MODY (Rothschild et al. 1992). The more distal markers, D20S43 and D20S55 (fig. 1*A*), have obligatory recombinations with MODY. These recombinations are supported by data from flanking markers. There is some encouragement that mapping may improve in the near future, since an additional large branch of the R.-W. MODY family has been identified. This branch segregates the same marker alleles linked to the early-onset form of NIDDM. In addition, another large family with chromosome 20–linked MODY has recently been identified (D. W. Bowden and E. Colle, unpublished results).

One of the 20q12-q13.1 markers, D20S22, could not be uniquely placed within the genetic map. This probably reflects the close proximity of D20S22 to the highly informative marker D20S16 (most likely distance is 1 cM; fig. 1*B*). Presumably because of the small size (1.4 kb; Stolz et al. 1991) of the clone for D20S22, we have been unable to unambiguously map it relative to D20S16 by using FISH, as has been done for the D20S16, D20S17, and ADA loci. In contrast, the PPGB locus was uniquely placed relative to the other markers. This was surprising, since PPGB and D20S17 (as revealed by D20S17 cosmids) are apparently found on the same yeast artificial chromosome clone (G. Gillespie, personal communication). The order of D20S17 and PPGB is based on one recombination event between them. This appears to be a genuine event, since the pattern of inheritance of flanking markers is consistent with a crossover occurring between D20S17 and PPGB.

One important question for efforts at identifying the MODY gene through positional cloning is the relationship of the physical and genetic map lengths. The physical distances around D20S16 may be shorter than would normally be assumed from the genetic distance. Several experimental approaches suggest that the D20S16 locus may be a recombination hot spot (D. W. Bowden, unpublished data). This possibility is consistent with results from FISH in which the D20S16 and D20S17 loci, which are genetically 4.8 cM apart (fig. 1), could not be seen as separate signals on metaphase chromosomes (fig. 2), but the D20S17 and ADA loci could be seen as separate signals on metaphase chromosomes even though the genetic distance between them is smaller (on the order of 2.3 cM; fig. 1). Tertiary structure of the chromatin in metaphase chromosomes in this region could, however, also be contributing to this difference.

Finally, the genetic map of 20q12-q13.1, constructed with the highly polymorphic loci and supplemented with physical mapping data from FISH for D20S16 (Rao et al. 1992), D20S17, and ADA, will also act as a framework for construction of a detailed physical map of the region, as a prerequisite for positional cloning of the MODY gene. We are adding additional markers to the region by identifying microsatellite polymorphisms associated with other genes in 20q12-q13.1 and by isolation of new markers from a library derived from a microdissected fragment of this region.

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