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Linkage Analysis of Maturity-Onset Diabetes of the Young (MODY): Genetic Heterogeneity and Nonpenetrance

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Summary

We have analyzed the inheritance of maturity-onset diabetes of the young (MODY) on chromosome 20 in a large multigeneration family, the R.-W. family, and in two other MODY families. Of the four branches of the R.-W. pedigree which have been studied, two have documented early onset of non-insulin-dependent diabetes mellitus (NIDDM), while there is no evidence of early onset in the other two branches. The early-onset branches have apparently inherited the same D20S16 allele from the affected parent, while another D20S16 allele was inherited in the two branches without evidence of early onset. A test for homogeneity, the *M*-test, using the results of two-point linkage analysis with D20S16 indicates heterogeneity between early- and late-onset branches of the R.-W. family ($P \leq .014$). In addition, analysis strongly suggests that MODY as expressed in the EDI and WIS families is unlinked to loci on chromosome 20 ($P \leq .018$–$.004$). Comparable results are seen when the data are analyzed by the HOMOG program. Three polymorphic loci—D20S16, D20S17, and ADA—show no recombination with the MODY locus when two-point linkage analysis is used in the early-onset branches of the family. The multipoint lod score in the early-onset branches of the R.-W. family is 10.16, with the most likely location being between D20S4 and D20S17. Multipoint linkage analysis using the CHROMPICS option of the program CRI-MAP has been used to follow inheritance of the MODY disease locus. This analysis has identified two cases of possible nonpenetrance in the early-onset branches of the family (odds of at least 156:1), as determined by the appearance of apparent isolated double crossovers at the MODY locus in these unaffected individuals.

Introduction

Non-insulin-dependent diabetes mellitus (NIDDM; type 2 diabetes mellitus, adult-onset-type diabetes) is one of the most common chronic disorders. On the order of 5% of adult Americans are affected with this disorder. Twin studies have suggested that genetic predisposition is an important component for developing the disease (Rotter and Rimoin 1980; Barnett et al. 1981), but the etiology of the disorder remains obscure. Because of the late age at onset, premature mortality, and clinical heterogeneity, NIDDM has been a difficult condition to analyze with genetic methods.

Recently, two groups, working independently and using different polymorphic markers and different assumptions in their linkage analysis, observed coinheritance of maturity-onset diabetes of the young (MODY), as expressed in one large family (the R.-W. family), with polymorphic loci on chromosome 20 (Bell et al. 1991; Bowden et al. 1992). Two branches of this large pedigree had documented expression of NIDDM at an early age (i.e., in many members at less

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than 25 years of age). Two other branches had no documentation of early-onset diabetes, and one of these branches has an individual in whom NIDDM was recognized after the age of 60 years. These apparent differences in age at onset and differences in the hormonal and metabolic expression of the disorder (Fajans et al., in press) suggested the possibility of heterogeneity. In addition, two apparently unaffected individuals in the early-onset branches of the family had, with chromosome 20 RFLP loci, inheritance patterns which suggested that they either were products of recombination events or were nonpenetrant for the disease. With the genotyping of additional family members and with the addition of genotypic data from more informative loci at D20S17 and the ADA *Alu*VpA polymorphism (Economou et al. 1990), we have used two-point and multipoint linkage analysis methods to investigate (1) genetic heterogeneity in the R.-W. family and in two other MODY families and (2) possible nonpenetrance in the R.-W. family.

Material and Methods

DNA Probes and Genotypic Data

Initially genotypic data were collected with nine polymorphic systems (eight loci) from chromosome 20 (Bowden et al. 1992). Additional genotypic data have been collected from the families with RFLP loci and with two independent polymorphic dinucleotide repeat loci isolated from cosmids containing the D20S17 locus. With these polymorphisms the cumulative PIC for the locus is now over .80 (D. W. Bowden, C. B. Rothschild, and G. Akots, unpublished data). The *Alu*VpA polymorphism (Economou et al. 1990), which has a PIC of .69, has also been genotyped in the families. The order of loci on the genetic map of chromosome 20 (from the distal short arm to the distal long arm) is D20S5, D20S6, D20S14, D20S18, D20S17, ADA, D20S16, D20S4, and D20S15. This order was determined from cytogenetic localizations and genetic mapping data (contributed to the CEPH data base, version 4) and was obtained, in our laboratories, for the D20S17 dinucleotide repeats (i.e., DWB) and for the ADA *Alu*VpA repeats (i.e., SEA) from genotyping on the CEPH families. Map construction was carried out in a manner described in detail elsewhere (Bowden et al. 1989) by using odds of 1,000:1 over any other order as the criterion for a unique order. The loci extend from D20S5 at p12 (Goodfellow et al. 1987) to D20S15 (distal to q13.2). D20S16, the most informative locus, revealed by CRI-L1214 (Donis-Keller et al. 1987), is a complex, site-rearrangement polymorphism (Schumm et al. 1988) with a PIC of .98 (Donis-Keller et al. 1987).

In addition to the 97 R.-W. family members studied previously (Bowden et al. 1992), we have genotyped an additional 18 family members. Four branches of the R.-W. family were studied. Two of the branches—II,2 and II,5—have multiple documented cases of early-onset NIDDM (under the age of 25 years) and have individuals with NIDDM in generations II–V. In contrast, two other branches—II,3 and II,6—have no individuals with recognized early onset of NIDDM. The II,3 family has NIDDM in generations II and III only, and II,6 has NIDDM in generation II only. For simplicity the II,3 and II,6 branches of the family will be referred to as "late-onset" NIDDM. It should be noted that for our purposes this means "no early onset" NIDDM. Two other MODY families have also been studied. The pedigrees of these families, called the EDI and WIS families, are shown in figure 1. Both families have early-onset, nonketotic, diabetes with evidence of autosomal dominant inheritance (segregation of the disease through at least three succeeding generations).

Analysis

In our earlier work we carried out calculations using the CRI-MAP linkage analysis program (Donis-Keller et al. 1987), which has the advantage of rapidly carrying out multipoint linkage analysis calculations simultaneously with large numbers of loci. In the case of the R.-W. family this was appropriate for calculations that limited the analysis to affected individuals only (i.e., unaffected individuals were coded as phenotypically unknown). Because of our interest in studying the apparently variable age at onset seen in the R.-W. family, we have now carried out both two-point and multipoint linkage calculations by using the program LINKAGE (version 4.8) (Lathrop et al. 1984), which has the advantage of being able to incorporate estimates of age-related penetrance, in addition to the ability of inferring genotypes of missing individuals in a pedigree.

Estimates of age-related penetrance were determined using a stepwise age-at-onset estimate and assuming complete penetrance. The terms "age of onset" and "age at diagnosis" in NIDDM can mean significantly different things. It is well documented that individuals can be affected with NIDDM, but if individuals are untested the disease may remain undetected for many years, even decades (Fajans 1989). For this

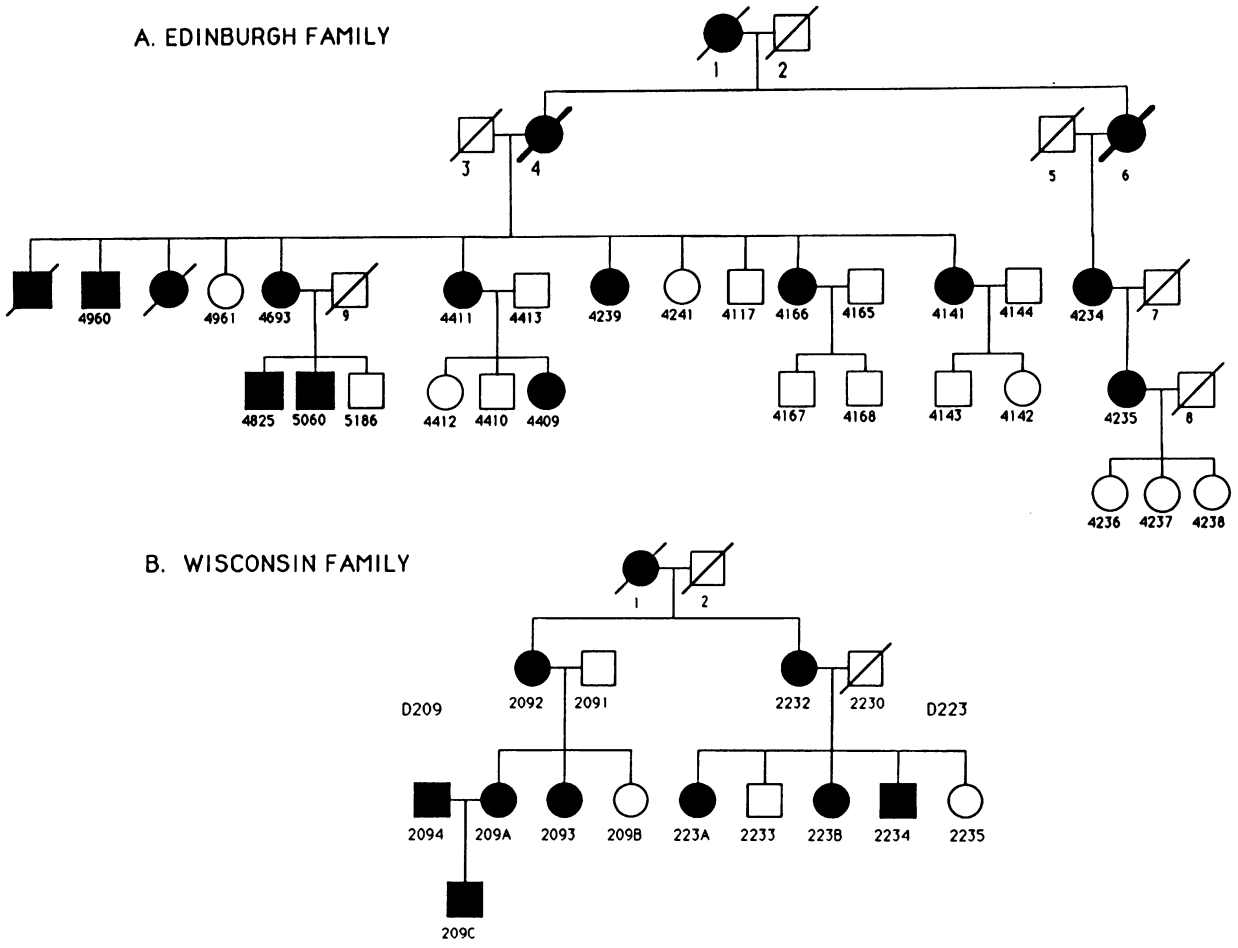
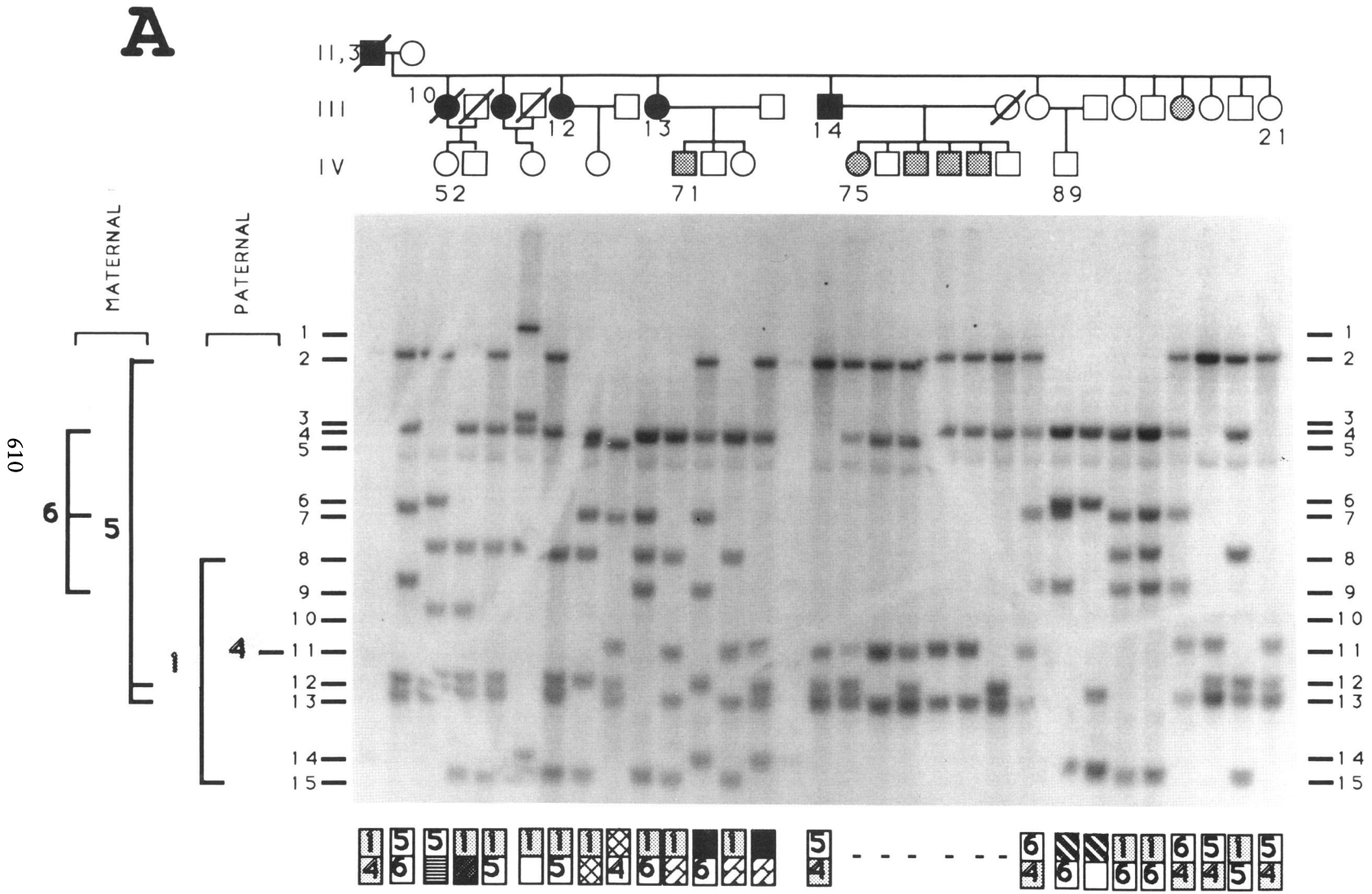


Figure 1 Pedigrees of WIS and EDI families

reason, we have based our age-related penetrance estimates on data from 19 individuals in the R.-W. pedigree in whom NIDDM was diagnosed during the course of the present study. Of these individuals, 17 are from the early-onset branches of the family. Five liability classes were used, and a dominant mode of inheritance was assumed. Ages and penetrance estimates for these classes were (1) 0–10 years, .07; (2) 11–20 years, .31; (3) 21–30 years, .64; (4) 31–40 years, .83, and (5) >40 years, .93. We have also included an estimate of 5% phenocopies (for cases due to non-chromosome 20–linked loci) for individuals over the age of 40 years. Spouses were given the same phenotype assignment as were clearly unaffected members of the families. Individuals in the pedigrees with impaired glucose tolerance were coded as having unknown phenotype. Disease-gene frequency in the population was set at 1%. Both two-point and multipoint linkage analysis were carried out. Distances between loci for the multipoint analysis were derived from mapping data on CEPH families.

Heterogeneity was assessed using the *M*-test described by Morton (1956). In brief, for the case of the R.-W. family, we have treated the family as two families (early and late onset), calculating maximum lod scores for each family individually and then with both the early- and late-onset branches simultaneously. The difference between the sum of individual Lod_{max} values for each family and the maximum lod score calculated for all branches together can be transformed to χ^2 through multiplication by $2(\ln_{10})$, and the appropriate *P* value can be read from χ^2 tables for an appropriate df. Heterogeneity was also assessed using the HOMOG (version 2.95) program (Ott 1985). Heterogeneity was defined as two family types: one type linked to chromosome 20 loci, and the other


A
II,3
III
IV
10
12
13
14
21
52
71
75
89
MATERNAL
PATERNAL
6
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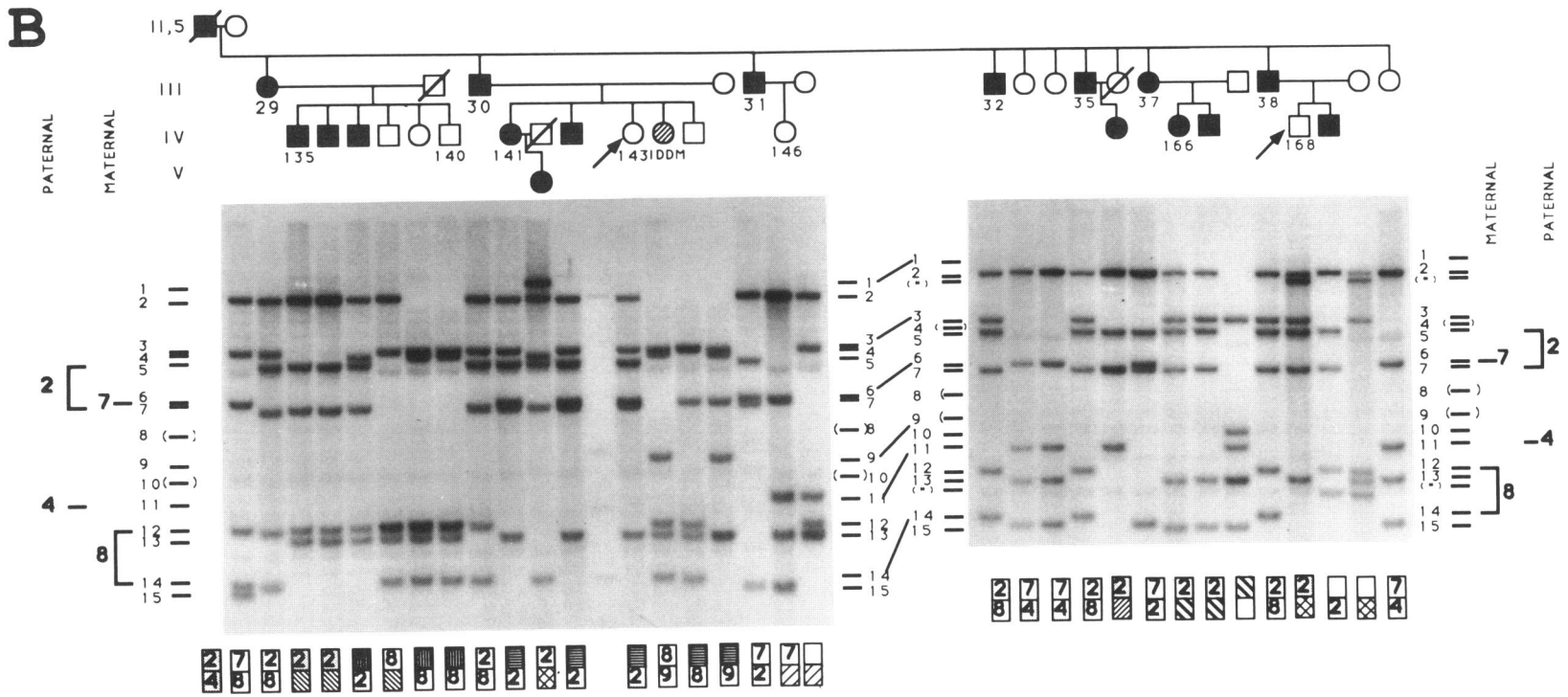


Figure 2 Inheritance of D20S16 alleles in II,3 and II,5 branches of R.-W. pedigree. Autoradiograms are of Southern blot hybridizations of radiolabeled D20S16 to genomic DNAs from the R.-W. family that have been digested with *Bgl*II restriction enzyme. Methods have been described in detail by Donis-Keller et al. (1987). Four generations of the pedigree are shown, starting with II,3 and II,5 at the top. Blackened symbols denote affected individuals; unblackened symbols denote unaffected individuals; and grey-stippled symbols denote individuals with impaired glucose tolerance. Pedigree numbers are shown below appropriate individuals in generations III and IV. Frequently hybridizing genomic DNA fragments are shown numbered on the side. Allelic sets of fragments are also noted. The paternally derived alleles (from individuals II,3 and II,5) are numbered in grey-stippled backgrounds. Allelic sets of fragments from the spouses in generation II are also shown. The noted allelic sets of fragments are not meant to encompass *all* the fragments which make up an allele; rather, they are the fragments which can unambiguously be used to follow inheritance. Allele determinations for each individual are shown at the bottom of the figure. For simplicity, allelic sets of fragments from spouses in generations III and IV are shown figuratively as grey-stippled, hatched, or lined boxes. *A,* D20S16 alleles from II,3 branch of family. *B,* D20S16 alleles from II,5 branch of family. Results are from two films, each showing part of the family. Arrows denote individuals for whom there is evidence of nonpenetrance of the MODY phenotype. Individual IV,144 has type 1 insulin-dependent diabetes. As noted in the text, IV,143 has one daughter with MODY. That individual was not genotyped on this Southern blot.

type was unlinked. The proportion of families which are linked is denoted α_1. With this set of genotypic data, H1 is the hypothesis for linkage homogeneity in the families, H2 is the hypothesis for linkage heterogeneity, and H0 is the hypothesis of no linkage.

Evaluation of nonpenetrance was aided by the use of the CHROMPICS option of the CRI-MAP linkage analysis program. Using the genotype data derived from mapping the polymorphic loci on the R.-W. family, and using the known order of the loci on chromosome 20, CHROMPICS shows the grandparental origin of informative loci for each individual in the pedigree. The program output specifies crossovers between adjacent loci and notes isolated double crossovers. Data from multiple flanking probes and sex-specific recombination fractions between loci can, in conjunction with this information, be used to estimate the likelihood that double-recombination events (or single-recombination events) have occurred in a specific interval (Donis-Keller et al. 1987).

Results

Different D20S16 Alleles Are Inherited in Branches of the R.-W. Family

As can be seen in figure 2, the highly polymorphic locus D20S16 (probe CRI-L1214) reveals complex, variable sets of genomic DNA fragments. Genomic DNA fragments of different sizes associate in multiple allelic combinations in different families. With many children and several generations in a family, as are present in the R.-W. MODY family or in mapping families from CEPH, the allelic combinations of fragments can be determined with a high degree of confidence. D20S16 is fully informative in the R.-W. family—except for the progeny of III,14 in which the alleles cannot be read without ambiguity (Bowden et al. 1992). The reading of these alleles for a late-onset branch (II,3) of the family and for an early-onset branch (II,5) are shown in figure 2*A* and *B*, respectively. Both late-onset branches (II,3 and II,6) inherited alleles A1/A4 (fig. 2*A*, II,6 data not shown), while one early-onset branch (II,5) has inherited alleles A2/A4 (fig. 2*B*) and the other early-onset branch (II,2) has inherited alleles A2/A3 (data not shown). These observations are consistent with (*a*) one allele, A2, being coinherited with early onset of the disease and (*b*) another allele, A1, being coinherited in branches with late onset of the disease. It was apparent, however, that many (over 10) unaffected individuals in the late-onset branches inherited A1 but were unaffected. These observations suggested some form of heterogeneity in the family. This possibility was investigated through linkage analysis.

MODY Heterogeneity: Two-Point Linkage Analysis

Table 1 shows the maximum lod scores and corresponding recombination fractions calculated for linkage of D20S16 and MODY. In the first case the early-onset and late-onset branches of the R.-W. family were treated as separate families. Lod scores were calculated using the MLINK and ILINK options of LINKAGE. Maximum lod scores and recombination fractions for the early- and late-onset branches individually, the sum of the maximum lod scores, and the maximum lod score for the early- and late-onset branches calculated simultaneously are shown. There is a striking difference between the lod scores for the

Table 1

Two-point Linkage Analysis: D20S16 and *M*-test

	Maximum Lod Score	Peak Recombination Fraction	χ^2	*P*
R.-W. family:				
Early-onset branches (II,2 and II,5)	10.16	.001	. . .	. . .
Late-onset branches (II,3 and II,6)	.00	.50	. . .	. . .
All R.-W. family branches	8.80	.001	6.26	⩽.014 (1 df)
EDI family	.00	.50	. . .	. . .
WIS family	.195	.20	. . .	. . .
R.W. (all branches), EDI, and WIS families	7.35	.05	13.84	⩽.004 (3 df)
R.-W. (early-onset branches), EDI, and WIS families	8.55	.05	8.31	⩽.018 (2 df)

early-onset branches (II,2 and II,5) and the late-onset branches. The *M*-test indicates that this difference is significant at $P \leqslant .014$. In the second case the *M*-test is again used to test for heterogeneity between MODY as expressed in the R.-W. family and as expressed in two other MODY families, the EDI and WIS families. There is evidence for heterogeneity, with $P \leqslant .004$. In the third case, the R.-W. early-onset, EDI, and WIS data were compared, and again there is evidence for heterogeneity ($P \leqslant .018$).

In addition to the *M*-test, we have analyzed the genotypic data obtained with D20S16 on the families by the program HOMOG (version 2.95). Results from this analysis are summarized in table 2. For simplicity these calculations treated the branches of the R.-W. family as separate families. (The II,6 branch of the family has only six typed members and consequently provides little data). Testing H2 versus H1 gives an odds ratio of greater than 30:1 for heterogeneity. In addition, the calculated α is consistent with our prior observations. Finally, the calculated conditional probabilities of linkage for each family are consistent with results of both the pattern of D20S16 allele segregation and the *M*-test: R.-W. early-onset families are linked, while the other families studied are unlikely to be linked.

In addition to two-point linkage analysis with D20S16, we have carried out two-point analysis with the genotypic data from the early-onset branches of the R.-W. family and with other chromosome 20 loci. Three loci—D20S16, D20S17, and ADA—show no recombination with MODY. These results are shown in table 3. For D20S16, which is fully informative in the families, the two-point analysis with the late-onset R.-W. branches and with the WIS and EDI families is also shown. While lack of recombinants prevents us

Table 2

Test for Heterogeneity by HOMOG

A. Results of Heterogeneity Test

Hypothesis	Estimates of: Maximum ln Likelihood	α_1	Recombination Fraction
H2	20.11	.40	.00
H1	16.69	(1)	.05
H0	(0)	(0)	(.50)

B. Components of χ^2

Components Compared	df	χ^2	*P*
H2 vs. H1, heterogeneity	1	6.84	.0045
H1 vs. H0, linkage	1	33.38	.0000
H2 vs. H0, total	2	40.22	.0000

C. Conditional Probabilities of Being Linked

Family	Conditional Probability of Linked Type	Approximate Confidence Limit: Upper	Lower
R.-W. II,2	.977	.967	.999
R.-W. II,3	.030	.002	.493
R.-W. II,5	1.000	1.000	.000
R.-W. II,6	.393	.049	.949
EDI	.011	.001	.780
WIS	.047	.004	.943

Table 3

Two-Point Linkage Analysis in MODY Families

	Lod Score at Recombination Fraction of									
	.00	.05	.10	.15	.20	.25	.30	.35	.40	.45
D20S16:										
R.-W. early-onset branches....	10.16	9.35	8.47	7.54	6.54	5.51	4.40	3.24	2.03	.89
R.-W. late-onset branches.....	−1.35	−1.30	−1.15	−.95	−.72	−.52	−.35	−.21	−.11	−.04
EDI family	−2.05	−.74	−.50	−.37	−.30	−.25	−.20	−.16	−.10	−.05
WIS family	−1.14	−.06	.12	.19	.19	.17	.13	.08	.03	.00
D20S17:										
R.-W. early-onset branches....	8.25	7.52	6.76	6.02	5.25	4.45	3.59	2.68	1.72	.76
ADA:										
R.-W. early-onset branches....	6.12	5.65	5.15	4.62	4.04	3.43	2.77	2.07	1.32	.59

from uniquely placing MODY, the magnitude of the lod score should eliminate any question that genuine linkage is being observed in the early-onset families.

Multipoint Linkage Analysis

We have extended the observations made with two-point linkage analysis by carrying out multipoint linkage analysis with the MODY families and the mapped polymorphic loci on chromosome 20. The results of this analysis carried out with the LINKMAP option of LINKAGE are shown in figure 3. Several different analyses are shown: multipoint analysis of MODY linkage to the R.-W. early-onset branches (II,2 and II,5), linkage to the late-onset branches (II,3 and II,6), and, in addition, the results of multipoint analysis with the EDI and WIS families. There is a striking difference between the early-onset branches of the R.-W. family and each of the following: the late-onset branches of the R.-W. family, the EDI family, and the WIS family. The maximum lod score for the early-onset branches is 10.16 at D20S16, with the highest likelihood placement being in the interval D20S17-D20S4. In contrast, in the same interval the maximum lod scores are −0.64, 0.44, and −0.08 for the R.-W. late-onset branches and the EDI and WIS families, respectively, with lod scores of −1.35, −2.05, and −1.14, respectively, at D20S16.

Determination of Nonpenetrance through Multipoint Linkage Analysis

Several unaffected individuals in the early-onset branches of the pedigree have almost certainly inherited the allele which leads to early onset of MODY. These cases are illustrated in figure 4. Initially there was a high likelihood that one individual, IV,143, inherited but has not expressed the MODY disease gene. This was inferred because she has a daughter who developed NIDDM at the age of 10 years and because she also had one diabetic-glucose-tolerance test when she was 16 years old (Fajans 1989). She has, however, been normoglycemic for the past 16 years (Fajans 1989). In addition to D20S16, D20S17, and ADA, IV,143 is also informative with several flanking loci, including D2014/D20S18 and D20S4, as is shown in figure 4. From the CHROMPICS analysis it can be seen that IV,143 has inherited alleles from one chromosome 20 of her NIDDM-affected paternal grandfather. This same chromosome was inherited in the other affected individuals in early-onset branches of the family. For IV,143 to be normal, a double-recombination event would have had to have taken place in her father (III,30), somewhere between D20S14 and D20S4 on the carrier chromosome. The probability of this happening is a function of the recombination fraction between markers flanking the disease locus. These distances are known from mapping of the loci on the CEPH families, and, in addition, since there is a sex-specific difference in rate of recombination, the appropriate sex-specific recombination fraction was used. Individual IV,143 inherited this chromosome from her father, so the male recombination fraction was used. Since it is not known within which interval between D20S14/D20S18 and D20S4 (D20S14/D20S18–D20S17–ADA–D20S16–D20S4) the MODY locus lies (fig. 3), calculations for each possibility were used. The male-specific recombina-

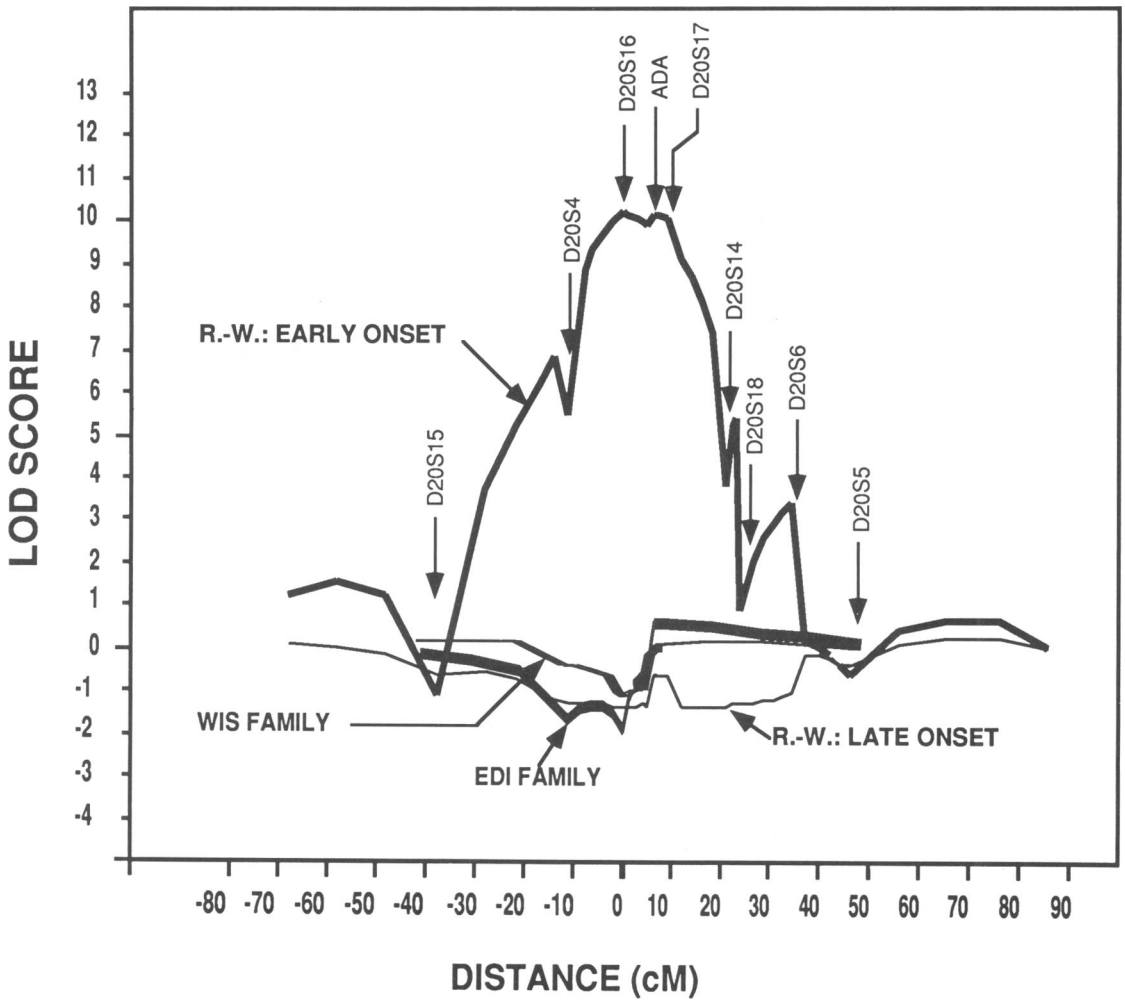
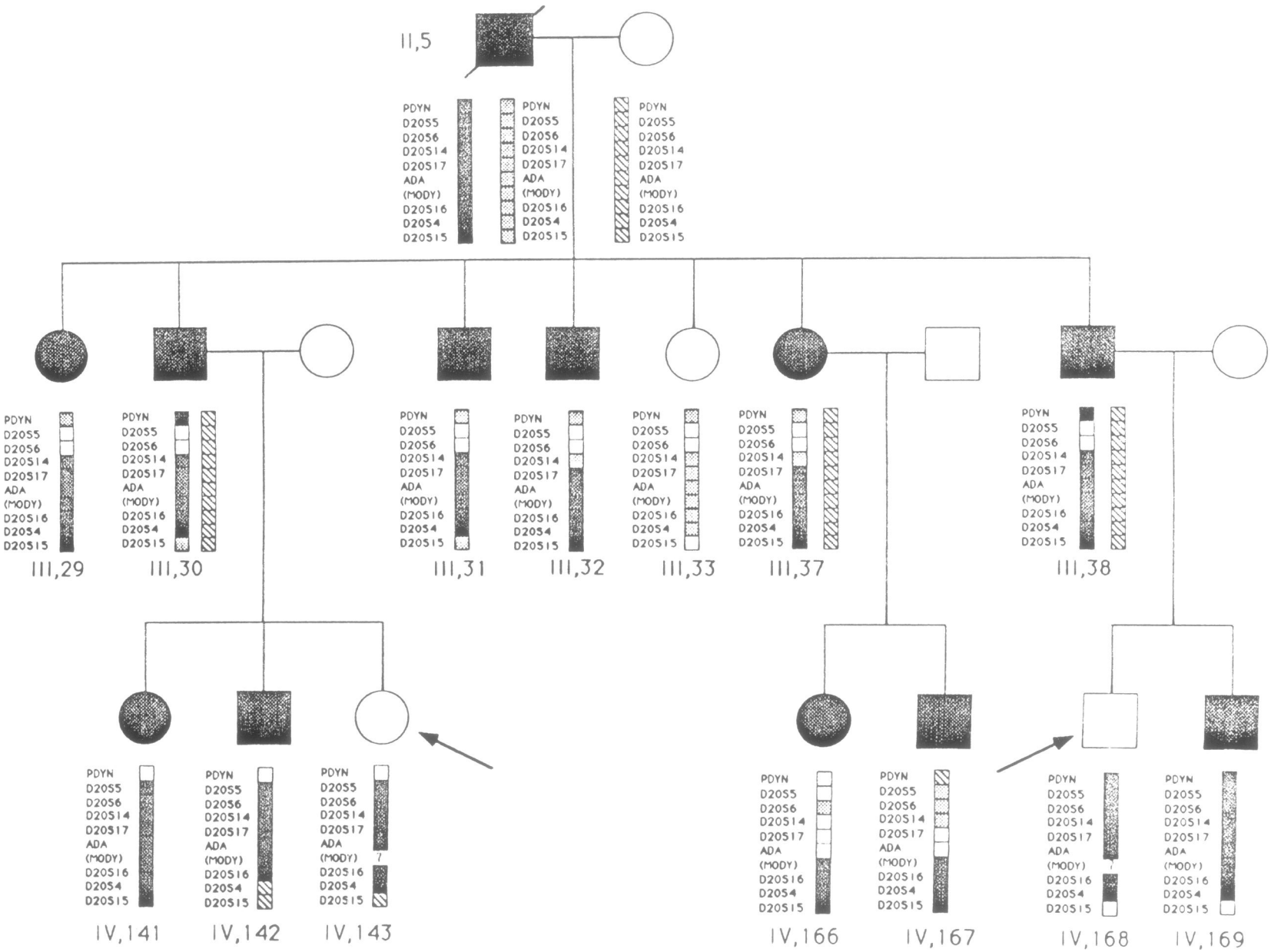


Figure 3 Multipoint linkage analysis of MODY with chromosome 20 RFLP loci. Lod scores were calculated, placing the MODY locus in each interval within the map of chromosome 20 RFLP loci. The arbitrary origin was chosen to be at D20S16, and distances (in cM) from this locus are shown. The order of RFLP loci was determined as described in Material and Methods. The order of D20S14 and D20S18 is not uniquely defined. Calculations are based on sex-averaged analysis assuming an autosomal dominant mode of inheritance. The distances (in cM) between chromosome 20 loci are as follows: pter–D20S5–11.7–D20S6–9.7–(D20S18, D20S14)–10.9–D20S17–2.1–ADA–6.5–D20S16–11.4–D20S4–27.7–D20S15–qter.

tion fractions for these intervals are .07, .05, .02, and .08, respectively. Consequently the likelihoods of a double crossover which places the normal MODY locus in these intervals on the inherited chromosome are .0049, .0025, .0004, and .0064 respectively. The odds against a double-crossover event taking place are at least 156:1, strongly suggesting that IV,143 inherited but has not expressed MODY. In like manner, IV,168 (present age 20 years) has the same likelihood (156:1) of inheriting the MODY disease locus: D20S14, D20S16, and D20S4 are informative, and MODY is transmitted through a male meiosis.

Discussion

Mapping of the MODY locus to chromosome 20 in the R.-W. family has permitted the investigation of several aspects of MODY inheritance. As outlined in tables 1 and 2 and figures 2 and 3, there is a high likelihood that NIDDM, as expressed within the R.-W. family and between MODY families, is heterogeneous in nature. In addition to the *M*-test, analysis with the HOMOG program (Ott 1985) gave similar results. The simplest view would be to assume that the early-onset branches (II,2 and II,5) of the R.-W.


II,5
PDYN
D20S5
D20S6
D20S14
D20S17
ADA
(MODY)
D20S16
D20S4
D20S15
III,29
III,30
III,31
III,32
III,33
III,37
III,38
IV,141
IV,142
IV,143
IV,166
IV,167
IV,168
IV,169
?

family have inherited a MODY gene on chromosome 20, while the other branches (II,3 and II,6) contain individuals affected through inheritance of a different NIDDM-conferring gene(s), as suggested by Bell et al. (1991). As we have noted elsewhere (Bowden et al. 1992), all five of the affected individuals in II,3 (late-onset branch) have, however, inherited the same (or same part of) chromosome 20, as judged by inheritance of D20S16 alleles. Since the different branches of the family have inherited different alleles of D20S16 (fig. 3), there exists the formal possibility that a second diabetogenic allele for the chromosome 20 MODY disease locus exists. This locus could lead to later onset of the disease, through a variety of possible mechanisms (Bowden et al. 1992).

In addition, our analyses have been based on age-at-onset diagnosis data collected through study of the R.-W. family for several decades. Ascertainment during a 30-year period could lead to preferential detection of early-onset cases compared with a form in which late age-at-onset is, for example, at the average age of 55 years. This is empirically true, since 17 of the 19 individuals on whom the penetrance estimate was based are from the early-onset branches of the family. That is, diabetes in all branches of the family could be linked to chromosome 20, but the average age at onset in the family is later than that inferred from the available data. This potential problem is intrinsic for any model in which projections of disease status are based on likelihoods rather than on definitive clinical observations. That is why we used an affecteds-only analysis in our initial description of this MODY linkage (Bowden et al. 1992).

Several lines of evidence suggest that the late-onset branches of the R.-W. family have not developed NIDDM as the result of inheriting the chromosome 20-linked MODY gene. First, neither the EDI family nor the WIS family shows evidence of linkage on chromosome 20, suggesting that it is unlikely that two different NIDDM alleles of the chromosome 20 locus would be segregating in the R.-W. family: the chromosome 20 MODY defect is not common in the population. In addition, for the late-onset families we have carried out calculations in which we have altered the age-related penetrance so that penetrance was reduced to 50% in individuals over 40 years old (and to proportionally lower levels in younger people). This would be the case either if late onset was due to a different MODY allele or if penetrance was reduced by coinheritance of some modifying factor. Two-point lod scores are still negative with D20S16 (maximum lod score 0 at recombination fraction .50, with the corresponding result in the M-test being $P \leq .014$) for the late-onset families, still suggesting heterogeneity. Finally, there appear to be differences in the hormonal and metabolic expression of the disease in the early- and late-onset branches of the R.-W. family (Fajans et al., in press).

As demonstrated in figure 4, with the use of informative flanking markers we can now predict whether individuals in the early-onset branches of the family have inherited the MODY disease allele. This will be important clinically for risk assessment and possible early intervention. In addition, it will be helpful in determining which factors, if any, prevent or delay the onset of NIDDM (e.g., in IV,143). It is interesting to note that subsequent to this analysis IV,143 had severe hyperglycemia during prolonged intravenous glucose infusion. Individual IV,168 had one impaired glucose tolerance at the age of 10 years but had normal glucose tolerance at ages 13, 14, 16, and 17 years. When recently retested at age 20 years, IV,168 had impaired glucose tolerance.

Our analysis using the LINKAGE program resulted in a different highest-likelihood placement of the MODY locus (on either side of D20S16; fig. 3) when compared with our previous analysis, with CRI-MAP, which placed the MODY locus proximal to D20S16 (Bowden et al. 1992). The difference is due to the assumptions made when carrying out the linkage analysis calculations. In the affected members of the R.-W. family there are two apparent crossover events between D20S16 and MODY. One of these occurs in an individual in the II,3 (i.e., late-onset) branch of the

Figure 4 Chromosome inheritance in branch II,5 of R.-W. family. Beneath each individual is a representation, based upon analysis with the CHROMPICS option of CRI-MAP, of chromosome 20. The dark-shaded parts of the chromosome indicate the portions of the early-onset MODY locus–carrying chromosome in each individual from II,5; the light-shaded portions of the chromosome originated with the sister chromosome 20 from II,5; and the hatched portions came from spouses. Unshaded portions indicate that the phase cannot be determined for that locus. For simplicity, only some of the typed individuals in II,5 are shown. In addition, only one chromosome is shown for the spouse of II,5, and only the chromosomes which are involved in the transmission of MODY are shown in succeeding generations. Arrows indicate individuals who are candidates for nonpenetrance of the MODY disease gene. The locus order is shown with MODY proximal to D20S16 (see text).

family and, on the basis of flanking markers, is undoubtedly a genuine recombination event (Bowden et al. 1992). As discussed above, we cannot prove that NIDDM in this branch of the family is due to a locus on chromosome 20. The other apparent recombinant is an individual in the II,2 (early-onset) branch. No flanking-marker information is available to confirm this crossover as genuine. The individual was diagnosed at age 48 years, so there is no proof that she has early-onset NIDDM. With CRI-MAP these crossovers are effectively considered genuine, while the assumptions made with LINKAGE reduce their influence on the gene placement. The situation may be resolvable through genotyping the II,2 family with additional linked, informative probes, which should help determine the origin of NIDDM. An examination of figure 4 would suggest that, in any event, the most likely position for the MODY locus is proximal to D20S16.

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