Identification of Genetic Markers Flanking the Locus for Maturity-Onset Diabetes of the Young on Human Chromosome 20

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A systematic search for genetic linkage with maturity-onset diabetes of the young (MODY) as expressed in the R.-W. pedigree has been carried out. Evidence for linkage was found with restriction-fragment–length polymorphism loci that map to human chromosome 20. Two-point linkage analysis with CRI-L1214 (D20S16) and MODY gave a log of the odds (lod) score of 4.16 at $\Theta = 0.08$. Multipoint linkage analysis with nine restriction-fragment–length polymorphism loci resulted in a lod score of 4.81 with the MODY locus in an ~20-cM region bounded by D20S16 and D20S14-D20S18. Examination of the pattern of MODY segregation suggests that additional factors, possibly genetic, could be involved in the age of onset of the disease. *Diabetes* 41:88–92, 1992

Non-insulin-dependent diabetes mellitus (NIDDM) is a common chronic disorder, affecting ~5% of adults in the United States. Despite extensive investigation, the origins of NIDDM are obscure. Studies in twins suggested a significant genetic component for developing NIDDM (1,2). Due to possible variable penetrance, late age of onset, premature mortality, and likelihood of heterogeneity, NIDDM has been a difficult condition to study genetically.

Families segregating maturity-onset diabetes of the young (MODY; 3,4) offer several potential advantages for studies of the genetic origin of NIDDM. Because of the early onset of the disease (frequently before 25 yr of age), multigenerational pedigrees have been identified that show expression of MODY in a pattern that suggests an autosomal dominant mode of inheritance (5,6). These pedigrees are useful for genetic linkage studies with DNA probes that reveal restriction-fragment–length polymorphisms (RFLPs). The R.-W. MODY pedigree (4,6,7; Fig. 1) has been the subject of extensive clinical characterization and potentially has enough family members to demonstrate linkage. The latter is an important advantage because there is considerable speculation that NIDDM could be genetically heterogeneous (4,6–8).

Until recently, linkage studies concentrated on the use of "candidate" genes. Several reports excluded linkage between MODY as expressed in the R.-W. family and genes coding for insulin on chromosome 11 (9), insulin receptor on chromosome 19 (10), and erythrocyte-HepG2 glucose transporter on chromosome 1 and apolipoprotein B on chromosome 2 (11). Recently, Bell et al. (12) described linkage of MODY in the R.-W. family with a polymorphic locus associated with the adenosine deaminase gene on chromosome 20. Independently, with different polymorphic markers and methods of analysis, we observed linkage with chromosome 20 RFLPs.

RESEARCH DESIGN AND METHODS

The R.-W. pedigree is shown in Fig. 1. Early onset is seen in branches II,2 and II,5 (4,6). There is no documentation of early onset (i.e., before 35 yr of age) of NIDDM in branches II,3 and II,6. It is important to distinguish between age of onset and age of diagnosis for NIDDM, because affected individuals (who have not been tested) may appear phenotypically normal for decades until routine blood glucose testing shows the disorder later in life, or the classical symptoms of diabetes or collateral complications of the disease become apparent (4,6). Accurate data on age of onset are available for only a fraction of the affected individuals in the pedigree. From these observations, we considered analyses with models

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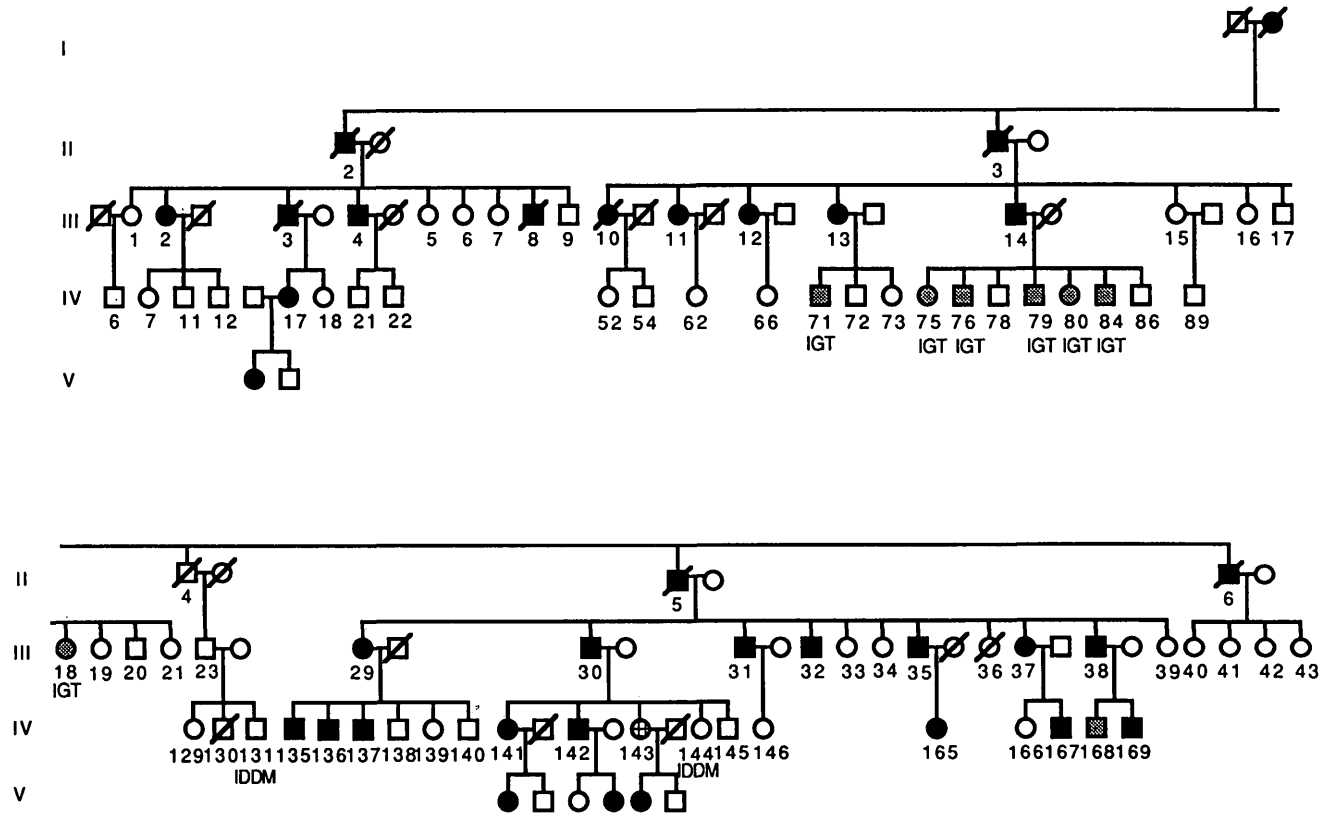

FIG. 1. R.-W. maturity-onset diabetes of the young pedigree. *Solid figures*, affected; *open figures*, unaffected; *shaded figures*, impaired glucose tolerance (IGT); *hatched circle*, IV,143. *Diagonal lines*, deceased or unavailable for study. IDDM, insulin-dependent diabetes mellitus.

that incorporate ad hoc estimates of age-related penetrance to be potentially misleading. The phenotype of unaffected individuals in the family cannot be confidently assigned. DNA from the 97 family members genotyped in this study included 28 affected individuals, 47 unaffected individuals, 8 individuals with impaired glucose tolerance (IGT), and 14 spouses. All available individuals in all branches of the family (II,2; II,3; II,4; II,5; and II,6) were used in our calculations. Individuals with IGT were coded unknown in the analyses. Subject IV,143 is unaffected (4,6) but has an affected daughter (diagnosis at age 10 yr). Consequently, IV,143 was coded unknown for the analysis. Only individuals used in this study are shown in Fig. 1. Assignment of diabetic phenotype was based on fasting plasma glucose concentration or the standard oral glucose tolerance test with National Diabetes Data Group (NDDG) criteria (13). Two individuals in the pedigree were diagnosed with IDDM (IV,131 and IV,144). These individuals were coded as unknown for the MODY phenotype in the linkage calculations.

Probes from chromosome 20 used in the analysis of MODY inheritance are listed in Table 1 as ordered on the chromosome: from the distal p arm to the distal q arm. This order was determined from the reported cytogenetic localizations, linkage analysis with the published data from the Centre d'Etude du Polymorphisme Humain (CEPH, Paris) version 3 database, and from segregation data obtained in the R.-W. family. Methods for map construction with CRI-MAP linkage analysis program (version 2.4) are described in detail by Donis-Keller et al. (14). The criteria used to establish locus order with linkage analysis was 100:1 (odds ratio) over any other order. For example, D20S14 has not been mapped on the CEPH families, but, based on the data from the R.-W. family, is 193-fold more likely to be between D20S6 and D20S17 than any other position on the chromosome. D20S14 and D20S18 are unseparated by recombination. The order of the loci that we determined is the same as mapped by Donis-Keller et al. (14). We used the map of markers on chromosome 20 constructed by Donis-Keller et al. and added markers PDYN and D20S14 (based on their highest likelihood placement) to make a new map. The new data do not change the Donis-Keller map. Methods for probe preparation, isolation of genomic DNA, restriction enzyme digestion, probe labeling, hybridization, and Southern-blot preparation are described in detail elsewhere (14). The restriction enzymes used, the polymorphism information content, and the cytogenetic localization (if known) are as follows: PDYN, *Taq*I/0.33, pter-p12 (15); D20S5, *Pvu*II/0.33, *Msp*I/0.34, p12 (16); D20S6, *Taq*I/0.37, p12 (16); D20S14, *Bam*HI/0.37, 20p (17); D20S18, *Msp*I/0.36 (14); D20S17, *Msp*I/0.33 (14); D20S16, *Bam*HI,*Bgl*II/0.98 (14); D20S4, *Msp* I/0.36, q13.2 (18); D20S15, *Pst* I/0.81 (14). Two-point linkage analysis (sex averaged) with MODY and chromosome 20 RFLP loci was carried out with CRI-MAP (14). CRI-MAP is particularly useful for rapid linkage calculations when many loci are being evaluated simultaneously. It is appropriate for calculations that are not based on age-related or incomplete penetrance, such as the affecteds-

TABLE 1
Two-point linkage analysis

Locus	Probe	Log of the odds (lod) score at recombination fraction							Maximum lod and recombination fraction	
		0.001	0.01	0.05	0.10	0.20	0.30	0.40	$\hat{z}$	$\hat{\Theta}$
PDYN	HDG1	−6.89	−3.91	−1.89	−1.09	−0.41	−0.13	−0.01	0	0.48
D20S5	pRI2.21	−3.29	−1.33	−0.07	0.34	0.54	0.44	0.22	0.54	0.20
D20S6	D3H12	0.90	0.88	0.79	0.68	0.45	0.23	0.07	0.90	0
D20S14	p4.8	−4.49	−0.74	0.46	0.81	0.86	0.61	0.27	0.89	0.16
D20S18	CRI-L1239	−0.13	0.37	0.91	1.00	0.85	0.54	0.20	1.00	0.10
D20S17	CRI-L127	0.60	0.30	0.28	0.26	0.20	0.15	0.08	0.30	0
D20S16	CRI-L1214	1.21	3.12	4.09	4.13	3.51	2.50	1.24	4.16	0.08
D20S4	pMS1-27	−2.69	−0.75	0.42	0.73	0.73	0.48	0.18	0.79	0.14
D20S15	CRI-L355	−8.39	−4.43	−1.81	−0.84	−0.12	0.08	0.07	0.09	0.34

$\hat{z}$, Maximum lod scores; $\hat{\Theta}$, corresponding recombination fractions. Unaffected individuals and individuals with impaired glucose tolerance were coded as phenotypically unknown for the analysis. Unaffected individuals marrying into the family were coded as phenotypically normal. Dominant mode of inheritance was assumed.

only calculations presented here. CRI-MAP does not weight allele frequencies to calculate likelihoods with missing individuals. This has only modest significance for the analyses reported here because the most important marker (D20S16) is fully informative in all but one mating in the entire family. CRI-MAP has previously been used in several linkage analysis studies with disease genes (19–21). Calculations were carried out as described previously (22), with phenotype assignments as described above. For multipoint linkage analysis, genotypic data collected with the R.-W. family were merged with any available published CEPH data for the calculations. The CEPH data are uninformative for MODY, but the substantial amount of segregation data allow more accurate determination of genetic distances between loci. Both multipoint and two-point analyses were carried out with CRI-MAP except where noted.

RESULTS

Because MODY could be due to a genetic defect for which a corresponding protein has not been identified or characterized, we systematically searched for linkage in the R.-W. family. Over 210 RFLP loci, detected by mapped sets of DNA clones from all 22 autosomes, have been genotyped (23,24).

On evaluation of linkage data obtained with RFLP loci from chromosome 20, we observed evidence for linkage to MODY when only affected individuals were used (coding unaffected family members as having unknown phenotype). Genotypic data from all available individuals were included in the calculations. Two-point linkage analysis of MODY with chromosome 20 probes is shown in Table 1. The highly polymorphic probe CRI-L1214 (D20S16) was informative for all meioses in the family except for the children of subject III,14. The maximum two-point log of the odds (lod) score for D20S16 and MODY, $\hat{z} = 4.16$ (at $\hat{\Theta} = 0.08$), exceeds the conventionally accepted lod score for evidence of linkage (lod ≥ 3). Other chromosome-20 probes with lower polymorphism information content values were less informative, reflecting, at least in part, fewer informative meioses. However, these lod scores were positive with the exception of the PDYN locus. Additional calculations carried out with the LINKAGE 4.8 program (25), including both affected and unaffected individuals with best-available estimates of age-related penetrance and frequency of phenocopies (5% > 45 yr of age), gave a two-point lod score (MLINK) of 4.39 ($\hat{\Theta} = 0$; 95% confidence interval $\Theta = 0–0.17$) for D20S16 and MODY and a multipoint lod score of 4.61. Because estimates of age of onset could

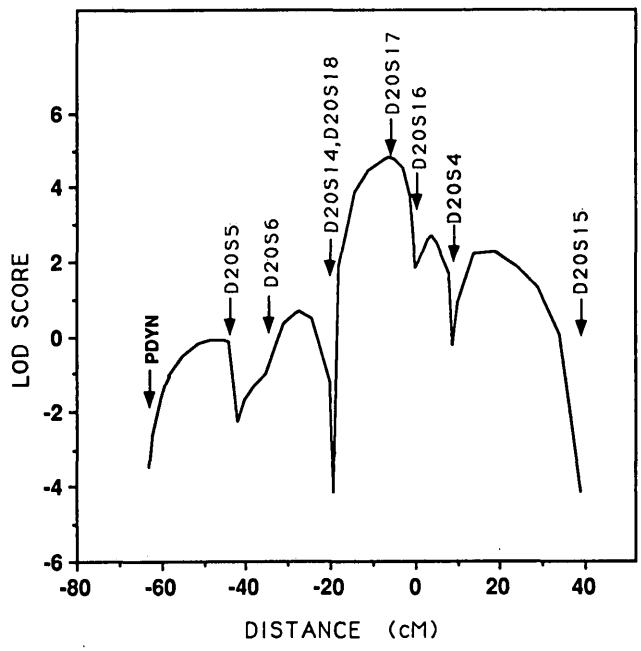

FIG. 2. Multipoint linkage analysis of chromosome 20 restriction-fragment-length polymorphisms (RFLPs) and maturity-onset diabetes of the young (MODY) in the R.-W. family. Ordered set of RFLP loci is as shown in Table 1. Locus D20S16 was chosen as arbitrary origin, and all genetic distances are in centimorgans from D20S16. Multipoint log of the odds (lod) score, calculated with CRI-MAP, for placement of MODY locus is shown. Distances (sex-averaged, cM) between loci, calculated with FIXED option of CRI-MAP (14,22) are as follows: PDYN←21.4→D20S5←4.2→D20S6←13.8→D20S18/D20S14←13.8→D20S17←5.4→D20S16←7.6→D20S4←28→D20S15.

be inaccurate, we used a more conservative analytical approach (the affecteds-only analysis) for the results generated with CRI-MAP (Table 1; Fig. 2).

Multipoint linkage analysis was carried out with all of the genotypic data obtained with chromosome 20 probes. The results of this analysis are shown in Fig. 2. The multipoint lod score for linkage to MODY was 4.81, with the most likely position for the MODY locus being in the interval between D20S14-D20S18 and D20S16 (odds ratio 131:1 over any other interval). This is a distance of ~20 cM (sex-average distance). D20S14-D20S18 and D20S16 are separated from the MODY locus by recombination events in two individuals. For D20S16, III,2 and III,14 are recombinants and for D20S14, III,32 and III,37 are recombinants. Three (III,14; III,32; III,37) of these four crossovers are consistent with the inheritance of more distal flanking markers (there are no data supporting or refuting the authenticity of the 4th crossover). CRI-L127 (D20S17) was poorly informative, but, based on the limited data, the MODY locus is closest to D20S17.

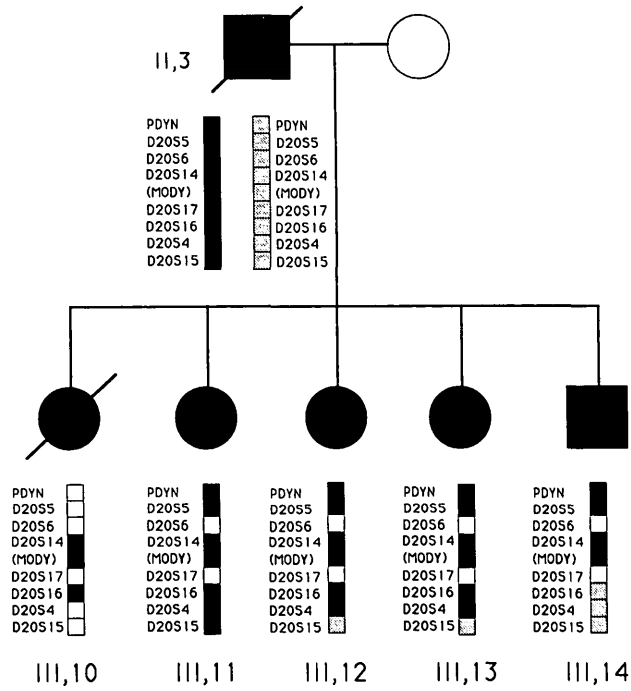

FIG. 3. Chromosome segregation in affected individuals from II,3. *Dark shading*, alleles derived from mother of II,3 (Fig. 1); *light shading*, alleles derived from father of II,3; *open symbols*, alleles of indeterminant origin. Phase assignments were computed with the Chrompics option of the CRI-MAP program with locus order as shown. Maturity-onset diabetes of young (MODY) refers to the locus rather than normal or affected alleles. Recombinants are seen between D20S15 and D20S4 in III,12 and III,13, and between D20S16 and MODY in III,14 (see RESULTS).

DISCUSSION

The linkage of MODY to chromosome 20 RFLP loci suggests many additional experiments. It is probably most important to test this linkage in other families that appear to segregate NIDDM (including MODY families) to assess the extent of heterogeneity of the disorder. Hormonal and metabolic differences among MODY pedigrees (4,6), such as differences in insulin secretion (hypoinsulinemia or hyperinsulinemia) and differences in insulin resistance may be due to differing genetic abnormalities. Preliminary results with two other early-onset, nonketotic families with CRI-MAP to calculate lod scores and the *M* test for homogeneity (26) suggest that they are not linked ($P \leq 0.01$) (D.B., unpublished observations). Unless a candidate gene on chromosome 20 turns out to be defective in the R.-W. family, it will almost certainly be essential to find other linked families to map the MODY locus to a more-limited region, a prerequisite for attempts to isolate the MODY gene by positional cloning.

The lack of uniform early onset of NIDDM in the R.-W. family leads to several observations. Variable age of onset is consistent with a high degree of variable penetrance in individuals who have inherited the MODY defect or with the possibility that two different diabetes-causing genes are segregating in the early- and late-onset branches of the family. Other possibilities are that different alleles (associated with differing ages of onset) of the same gene are segregating in the family or that modifying genetic determinants are segregating in the R.-W. family. Such a determinant or determinants, in conjunction with the chromosome 20 locus, could lead to early-onset NIDDM, i.e., MODY, as seen in the II,2 and II,5 branches of the family or late onset of the disease as documented in the II,3 branch of the family.

Chromosome inheritance in the II,3 (i.e., the potentially late-onset branch of the family) is consistent with (but does not prove) linkage of NIDDM to the chromosome 20 locus (Fig. 3). All five affected individuals (III,10–III,14) in the II,3 branch show inheritance of the same part of the paternally derived chromosome 20 carrying the MODY locus. One individual, III,14, shows recombination between MODY and CRI-L1214 (D20S16). The pattern of inheritance of distal markers is consistent with III,14 being the product of an authentic crossover between D20S16 and the MODY locus.

In summary, we demonstrated linkage between polymorphic loci on chromosome 20 and the MODY locus as expressed in the R.-W. family. The polymorphic loci flank the MODY locus, thereby defining the maximum size of the chromosomal segment that could contain the MODY gene. In addition, the flanking markers will allow accurate assessment of the carrier state of unaffected individuals in the pedigree. Finally, evidence has been presented that NIDDM expressed in the II,3 branch of the family, which shows no evidence of early onset of the disease, may also segregate with loci on chromosome 20.

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Immortalized cell lines from the MODY pedigree are stored at the National Institute of General Medical Sciences Mutant Cell Repository.

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