Rapid Publication

Linkage Analyses of the MODY3 Locus on Chromosome 12q With Late-Onset NIDDM

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Non-insulin-dependent diabetes mellitus (NIDDM) is a clinically and genetically heterogeneous disorder. Maturity-onset diabetes of the young (MODY), an autosomal dominant form of NIDDM, has been used as a model for genetic studies of NIDDM. We recently reported linkage between markers on chromosome 12q and diabetes in 25% of our French MODY families. To evaluate if this gene is also implicated in late-onset NIDDM, we performed linkage studies between two markers of the MODY region and diabetes in 172 families with late-onset NIDDM. Both parametric and nonparametric methods were used in a total of 600 affected sib-pairs. Linkage was rejected in this population by all methods, implying that the MODY gene on chromosome 12q is not a major gene for late-onset NIDDM in this population. However, we cannot exclude a modifying role in a polygenic disorder or an important role in some families. ***Diabetes*** **44:1243–1247, 1995**

Non-insulin-dependent diabetes mellitus (NIDDM) is a clinically and genetically heterogeneous disorder. With the exception of relatively rare early-onset forms that show autosomal dominant or mitochondrial inheritance, NIDDM appears to be polygenic in basis, and no genetic susceptibility factors have been identified to date that account for an appreciable proportion of the incidence of NIDDM. To overcome the difficulties of mapping genes in the polygenic forms of NIDDM, maturity-onset diabetes of the young (MODY), an autosomal dominant form of NIDDM, has been used as a model to identify diabetes-susceptibility genes. So far three MODY genes have been mapped. Mutations in a not as yet identified gene on chromosome 20q (1) result in impaired insulin secretion that leads to severe hyperglycemia (2). Linkage between NIDDM and this locus has been found only in one branch from a MODY family of German origin (the RW family) (1) but not in British or Italian late-onset NIDDM families (3). Mutations in the glucokinase gene on chromosome 7p are responsible for a mild form of hyperglycemia in MODY families (56% in France) (4,5), but are very rare in late-onset NIDDM (6,7). Recently, we demonstrated that a third MODY locus is tightly linked to markers on chromosome 12q in a region spanning 7 cM (8). Clinical characteristics of patients having mutations in this as yet not identified gene resemble those seen in late-onset NIDDM patients, suggesting that this gene might also play a role in the development of common forms of NIDDM.

We therefore examined this region in families with late-onset NIDDM. Here we report the results of linkage analyses for the MODY3 locus on chromosome 12q in 172 French families with late-onset NIDDM using parametric and nonparametric methods.

RESEARCH DESIGN AND METHODS

Families were ascertained through a multimedia campaign to identify diabetes-prone families for genetic studies. Among the 550 families identified, 172 were selected for linkage studies because of the presence of NIDDM in at least two siblings (a total of 187 sibships). For linkage analyses, all available siblings (affected and nonaffected) as well as the parents (when available) were included: 14 families with both parents and 37 families with one parent available. The study included 751 subjects of whom 573 were considered affected. Most of the sibships were of small size with 2 to 4 subjects (76%), with the largest sibship including 13 individuals. Clinical data were obtained for each subject during a standardized clinical examination performed at the Endocrinology Department of the Hôpital Saint-Louis, Paris, or by the subject's personal physician. Subjects were given a standard 75-g oral glucose tolerance test (OGTT) (n = 260) or, when glucose tolerance testing was not possible, fasting plasma glucose samples were obtained (n = 488). Of the diabetic subjects, 88% were being treated with oral hypoglycemic agents or insulin.

Diagnosis. Individuals were considered affected if they were being treated for NIDDM, if the results of the OGTT showed them to have diabetes or impaired glucose tolerance (IGT) according to World Health Organization criteria, or if they had a fasting plasma glucose value of >6.1 mmol/l on two separate measurements. Using these criteria, 466 of the affected individuals had overt NIDDM, 44 had IGT, and 63 had mild fasting hyperglycemia (FH). A total of 169 individuals were considered as normal, 55% of whom had had an OGTT; 3 were considered unknown; and 6 had insulin-dependent diabetes mellitus (IDDM) and were considered as unknown for linkage analyses. Except for the parents that were available for clinical testing, affected status of parents was reported by their offspring. Accordingly, in 19 nuclear families, both parents were reported as being affected, and in 100 families, one parent was.

Genetic studies. DNA was isolated from peripheral lymphocytes using standard procedures. Subjects from the chosen families had previously been screened for the A to G transition at nucleotide pair 3243 of the mitochondrial DNA encoding the $tRNA^{LEU(UUR)}$ gene to exclude families with this genotype (9).

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BMI, body mass index; FH, fasting hyperglycemia; IBD, identical-by-descent; IDDM, insulin-dependent diabetes mellitus; IGT, impaired glucose tolerance; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; MR, morbid risk; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; STRP, simple tandem-repeat polymorphism.

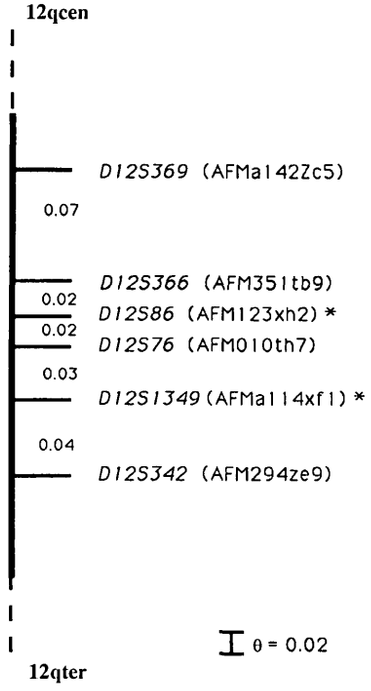

FIG. 1. Genetic map of the chromosome 12q region that contains the MODY3 locus. The two markers analyzed are indicated with asterisks. The sex-average recombination fractions (θ) between adjacent markers have been estimated from the Centre d'Etude du Polymorphisme Humain reference families.

Genetic markers. The MODY3 gene is localized in a region of chromosome 12q22-qter that spans 7 cM between D12S86 and D12S342, with a maximum location score obtained at the D12S76. Two simple tandem-repeat polymorphisms (STRPs) were chosen to be genotyped in NIDDM families to cover the MODY region (Fig. 1). The AFM123xh2 marker, corresponding to the D12S86 locus, is located 2 cM centromeric to D12S76 (10), whereas AFMa114xf1 at the D12S1349 locus lies 3 cM telomeric to D12S76 (J. Weissenbach, personal communication). The two STRPs were genotyped using a polymerase chain reaction (PCR)-based method with primers 123xh2-A (5′-AGCTAGTCTGGCATGAG-CAG-3′) and 123xh2-B (5′-CTATCCCCTGATGATCTCCC-3′) for the D12S86 locus and a114xf1-1 (5′-CCCAAGAGTGACATTGGC-3′) and a114xf1-2 (5′-GCATGGCTGGAGCATAAG-3′) for the D12S1349 locus. The PCR reactions were conducted in a final volume of 25 μl using unlabeled primers according to conditions ascertained by Genethon (10). The resulting PCR-products were separated by electrophoresis on a denaturing 5% polyacrylamide 8 mol/l urea gel, using a multiple loading procedure, blotted onto Hybond N+ nylon membranes (Amersham, U.K.) and hybridized against a nonradioactively labeled CA_{15} primer (ECL fluorescent labeling system, Amersham, U.K.) as described elsewhere (11). In order to reduce allele reading errors, autoradiograph films were read double blind and data entry into the database was standardized.

Linkage analyses

Logarithm of odds (LOD) score calculations. LOD score analyses were performed using the LINKAGE package of programs (12). Because the mode of inheritance of NIDDM is unknown, linkage was investigated under different genetic models (6). All models assume that family transmission of the disease is due to a single gene with two alleles D and d, where D is the allele increasing the susceptibility to NIDDM, as is classically done in linkage analysis. A monogenic model is specified by frequency q of the susceptibility allele D and the penetrance of the three genotypes (f_{DD}, f_{Dd}, f_{dd}), where the penetrance for a genotype is the probability of being affected given that genotype. Two modes of inheritance were considered for NIDDM: dominant, where the age- and sex-dependent penetrances f_{DD} and f_{Dd} are equal, and codominant, where the penetrance for the heterozygote is between those of the two homozygotes. For each assumed mode of inheritance of NIDDM, we considered two opposite situations, which can enclose a wide range of possible genetic models. These two situations are *1*) a rare susceptibility gene with a high lifetime penetrance and high rate of phenocopies and *2*) a more frequent susceptibility gene with a moderate lifetime penetrance and low rate of phenocopies. The parameter values at the disease locus, gene frequency and penetrances, were chosen to satisfy the age- and sex-specific cumulative morbid risks (MRs) in the French population (13). The cumulative MR is defined as the probability for a person to be affected with NIDDM at a given age. For a given age and sex class, the MR risk is calculated by $MR = q^2 f_{DD} + 2q(1 - q)f_{Dd} + (1 - q)^2 f_{dd}$. We assumed three age classes, <35, 35–55, and >55 years of age, with corresponding cumulative MRs of 0.5, 2.4, and 6% in males and 0.5, 2, and 4 in females. Table 1 presents the parameter values for each genetic model.

In addition, we used the genetic model in which linkage between NIDDM and chromosome 12 markers was initially shown in the French MODY families (8). This model assumes a frequency for the disease allele of 0.001 and equal female-to-male recombination rates. Four age-dependent liability classes were defined, <10, 10–25, 25–40, and >40 years of age, with age-related prevalences P of 0.0003, 0.0006, 0.0012, and 0.0025, respectively. The frequency of phenocopies among the affected subjects was assumed to be 0.5, 1, 10, and 30%, for the four age-dependent liability classes respectively, and was used to assign the penetrances for the homozygous nonsusceptible genotypes f_{dd} (0.0000015, 0.000006, 0.00012, and 0.00075, respectively). The pen-

TABLE 1
Genetic models assumed in linkage analyses of NIDDM

Parameters under each model	Age- and sex-specificity liability classes					
	Males			Females		
	<35 years	35–55 years	>55 years	<35 years	35–55 years	>55 years
DOM 1						
f_{DD}	0.20	0.63	0.90	0.20	0.50	0.60
f_{Dd}	0.20	0.63	0.90	0.20	0.50	0.60
f_{dd}	0.001 (20)	0.013 (50)	0.043 (70)	0.001 (20)	0.01 (50)	0.029 (70)
DOM 2						
f_{DD}	0.05	0.25	0.59	0.05	0.20	0.39
f_{Dd}	0.05	0.25	0.59	0.05	0.20	0.39
f_{dd}	0.00003 (0.5)	0.00055 (2)	0.0033 (5)	0.00003 (0.5)	0.00044 (2)	0.0022 (5)
COD 1						
f_{DD}	0.09	0.40	0.72	0.09	0.32	0.48
f_{Dd}	0.05	0.20	0.36	0.05	0.16	0.24
f_{dd}	0.00055 (10)	0.0055 (20)	0.0266 (40)	0.00055 (10)	0.0044 (20)	0.0177 (40)
COD 2						
f_{DD}	0.06	0.28	0.66	0.06	0.23	0.44
f_{Dd}	0.02	0.07	0.17	0.02	0.06	0.11
f_{dd}	0.00003 (0.5)	0.00069 (2)	0.00415 (5)	0.00003 (0.5)	0.00055 (2)	0.00277 (5)

Data are penatrances (% of phenocopies among affected individuals). DOM, dominant mode of inheritance; COD, codominant mode of inheritance. For DOM 1, DOM 2, COD 1, and COD 2, q = 0.01, 0.05, 0.05, and 0.15, respectively.

TABLE 2
Distribution of sib-pairs in the 172 NIDDM families

Number of possible pairs in the sibship (number of affected individuals)	Affected status							
	Overt NIDDM	Overt NIDDM + IGT + FH	Overt NIDDM & BMI <27	Overt NIDDM + IGT + FH & BMI <27	Overt NIDDM & BMI >27	Overt NIDDM + IGT + FH & BMI >27	Overt NIDDM & age at diagnosis <45 years	Overt NIDDM + IGT + FH & age at diagnosis <45 years
1 (2)	116	91	53	48	47	49	42	47
3 (3)	41	54	10	25	11	19	6	11
6 (4)	8	25	2	6	1	4	3	5
10 (5)	5	11	1	2	0	2	0	2
15 (6)	1	3	0	1	0	0	0	0
21 (7)	0	3	0	0	0	0	0	0
Total of sibships	171 (352)	187 (621)	66 (105)	92 (194)	59 (86)	74 (150)	51 (78)	65 (130)

Data are number of sibships (total number of sib-pairs). Affected sib-pairs were divided into eight subgroups according to their affected status, BMI, and age at diagnosis. The number of possible pairs in the sibship (n) is given by the formula $n = r\ (r - 1)/2$ where r is the number of affected individuals. The total number of sib-pairs in the sibships (N) in each subgroup is given by ΣnixNi.

etrances used for the homozygous and heterozygous susceptible genotypes f_{DD} and f_{Dd} in each liability class were 0.15, 0.30, 0.54, and 0.88, respectively.

Sib-pair analyses. Because the mode of inheritance for NIDDM is unclear, the nonparametric affected–sib-pair method was also applied for linkage analysis. This method of analysis, as described by Elston (14), compares the mean proportion of marker alleles identical-by-descent (IBD) estimated among affected sib-pairs with those expected on the basis of no linkage using a one-sided t test. In the absence of linkage, the mean proportion of alleles IBD in affected pairs should equal 0.5, whereas it should be greater than 0.5 if the marker is linked to the disease. The affected status was defined in either a narrow sense (overt NIDDM) or a broad sense (all affected, including NIDDM, IGT, and FH). Since NIDDM is clinically and genetically heterogeneous, we divided our panel of sib-pairs into more homogeneous subgroups, according to the body mass index (BMI) status or the age at diagnosis of NIDDM. Eight groups of sib-pairs were designed: *1*) all affected, *2*) all overt NIDDM, *3*) affected and lean (BMI <27 kg/m^2), *4*) overt NIDDM and lean, *5*) affected and obese (BMI >27 kg/m^2), *6*) overt NIDDM and obese, *7*) affected and age at diagnosis <45 years, and *8*) overt NIDDM and age at diagnosis <45 years. Because this susceptibility gene has been shown to be implicated in MODY, it may also be responsible for an earlier onset of disease in late-onset NIDDM. Therefore, we chose an age of 45 years, which is about 5 years below the average age of onset for NIDDM. Sib-pair analyses were carried out with the computer program SIBPAL of the S.A.G.E. package (R.C. Elston, J.E. Baily-Wilson, G.E. Bonney, B.J. Keats, A.F. Wilson, unpublished observations) (available from the Department of Biometry and Genetics, Louisiana State University Medical Center, New Orleans, LA). Calculations were made using all possible sib-pairs in each group. Table 2 describes the different subgroups of NIDDM families in which linkage analyses were performed. The 172 families include 171 different sibships with at least one sib-pair having overt NIDDM and 187 sibships with at least one affected (overt NIDDM, IGT, or FH) sib-pair. The maximum number of affected sib-pairs within this sample is 352 when affected status is overt NIDDM and 621 when affected status comprises overt NIDDM, IGT, and FH.

Allele frequencies were computed from the data by drawing one individual from each family at random.

RESULTS

The two markers, AFM123xh2 and AFMa114xf1, were genotyped in 751 members of 172 NIDDM families. They showed 25 and 13 alleles respectively with 93% and 71% heterozygosity.

Linkage studies. Linkage analyses were performed using parametric and nonparametric methods. Table 3 shows the results for LOD score calculations under the five genetic models we used. The total LOD scores for the two markers in the MODY3 region over all 172 NIDDM families were <-2 at recombination fractions up to $\theta = 0.05$ for all models, rejecting linkage between late-onset NIDDM and the MODY3 locus in this cohort of families. Because of the small sizes of the families, none of the individual LOD score values obtained in any of the 172 NIDDM families reached the significance level necessary to either exclude (LOD score <-2) or show evidence of linkage (LOD score >3) (data not shown).

Linkage results of sib-pair analyses in all subgroups of

TABLE 3
Pairwise LOD scores (Z) for late-onset NIDDM in 172 families versus the markers D12S86 and D12S1349 at MODY3 locus under different genetic models

Model	θ							
	0.00	0.001	0.01	0.05	0.1	0.2	0.3	0.4
D12S86 locus versus NIDDM								
Dominant 1	−31.62	−31.17	−27.57	−17.02	−9.54	−2.34	0.05	0.30
Dominant 2	−22.74	−22.44	−20.00	−12.48	−6.95	−1.62	0.07	0.21
Codominant 1	−11.05	−10.93	−9.92	−6.44	−3.62	−0.78	0.08	0.11
Codominant 2	−7.32	−7.24	−6.60	−4.28	−2.31	−0.32	0.19	0.10
MODY type	−124.46	−114.68	−80.58	−39.82	−21.06	−5.66	−0.62	0.30
D12S1349 locus versus NIDDM								
Dominant 1	−27.12	−26.80	−24.21	−16.01	−9.62	−2.91	−0.40	0.13
Dominant 2	−15.66	−15.48	−13.96	−9.05	−5.24	−1.34	−0.01	0.15
Codominant 1	−7.69	−7.61	−6.96	−4.65	−2.68	−0.61	0.05	0.09
Codominant 2	−5.69	−5.64	−5.16	−3.40	−1.85	−0.26	0.16	0.09
MODY type	−100.81	−94.37	−70.44	−37.44	−20.58	−6.08	−1.03	0.14

Total LOD scores (sum of all families) are shown at each recombination fraction under genetic models described in METHODS and Table 1.

TABLE 4
Sib-pair linkage analyses at the MODY3 locus in 172 NIDDM families

	D12S86 locus		D12S1349 locus	
	Affected sib-pairs	*P* value	Affected sib-pairs	*P* value
All affected	496 (0.52 ± 0.3)	0.059	586 (0.50 ± 0.29)	0.381
All NIDDM	302 (0.52 ± 0.31)	0.053	335 (0.51 ± 0.28)	0.129
Affected & BMI <27	157 (0.51 ± 0.3)	0.232	185 (0.51 ± 0.28)	0.308
NIDDM & BMI <27	93 (0.49 ± 0.31)	NS	101 (0.51 ± 0.28)	0.246
Affected & BMI >27	106 (0.51 ± 0.33)	0.297	145 (0.52 ± 0.31)	0.219
NIDDM & BMI >27	67 (0.55–0.34)	0.104	84 (0.55 ± 0.30)	0.054
Affected & age at onset <45 years	101 (0.5 ± 0.31)	0.451	123 (0.49 ± 0.32)	NS
NIDDM & age at onset <45 years	69 (0.52 ± 0.32)	0.266	75 (0.52 ± 0.32)	0.290

Data are *n* (mean proportion of alleles IBD ± SD). All affected includes NIDDM, IGT, and FH.

NIDDM families are shown in Table 4. No evidence for linkage between the MODY3 markers and diabetes was found in any of the subgroups.

DISCUSSION

A third MODY gene has recently been localized on the long arm of chromosome 12 (8); it might therefore be a candidate gene for late-onset NIDDM. The gene is located in a region spanning 7 cM, and we used two highly polymorphic markers 5 cM apart within this region for linkage studies. No evidence for linkage was found using either parametric or nonparametric methods between the MODY gene on chromosome 12q and diabetes in our set of French late-onset NIDDM families. Because gene parameters are not well known in the late-onset forms of NIDDM, we used five genetic models that assume an autosomal dominant or a codominant transmission for LOD score calculations. Linkage was rejected under all models. In addition, we performed nonparametric linkage analyses such as the sib-pair method that has previously been successfully used to map genes in complex diseases such as hypertension (15) or IDDM (16). Because NIDDM is clinically heterogeneous, we tried to sort our NIDDM patients into more homogeneous subgroups to enhance the chance to detect a true linkage. It is reasonable to postulate that a MODY gene is more likely to be found in lean NIDDM patients with an earlier age at diagnosis than in obese NIDDM patients with an older age at diagnosis. However, there was no evidence for linkage with the MODY3 locus, even in subgroups where diabetic subjects are lean or diagnosed before 45 years of age.

We did not find evidence for linkage at the MODY locus on chromosome 12q by using a combination of several linkage analyses, implying that this MODY gene is not a major gene for the common form of NIDDM in this population. However, it is possible that diabetes in some families is due to molecular alterations of this gene. In three families, the LOD scores between chromosome 12q markers and diabetes were close to their maximum attainable LOD scores, which were not enough to assess linkage because of the small size of the families (data not shown). Although those results can be expected from chance alone, they may indicate a linkage to the MODY locus in those families. That may especially be the case in one family in which an individual had diabetes diagnosed at the age of 21 years, suggesting that he might belong to an undiagnosed MODY family. In conclusion, the MODY gene on chromosome 12q is not a major gene for late-onset NIDDM, but mutation screening of the gene—once it is identified—will provide a clue of its exact role in some families.

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