

Novel Susceptibility Gene for Late-Onset NIDDM Is Localized to Human Chromosome 12q

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NIDDM has a substantial genetic component, but the nature of the genetic susceptibility is largely unknown. Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of NIDDM characterized by an early age of onset and autosomal dominant inheritance, and linkage studies have identified genes that are mutated in different MODY pedigrees on chromosome 20 (*MODY1* locus, hepatocyte nuclear factor-4 α [HNF-4 α] gene), chromosome 7 (*MODY2* locus, glucokinase gene), and chromosome 12 (*MODY3* locus, HNF-1 α gene). We studied an extended pedigree in which multiple members are affected by late-onset NIDDM associated with insulin resistance and performed linkage analysis with four microsatellite markers in the *MODY3* region of chromosome 12q. We found significant evidence for linkage between NIDDM and the *MODY3* locus (logarithm of odds score 3.65 at $\theta = 0.008$ telomeric to marker D12S321), but sequencing of the 10 exons and promoter of HNF-1 α did not identify any causative mutation in this gene. Our results indicate that the region of chromosome 12q close to *MODY3* harbors a novel susceptibility gene or genes for NIDDM. *Diabetes* 47:1793–1796, 1998

NIDDM is characterized by hyperglycemia caused by impaired insulin secretion, insulin resistance in muscle, and elevated hepatic glucose production (1). The causes of NIDDM are poorly understood, but its familial clustering and high rate of concordance in monozygotic twins implicate genetic factors (2). It is a genetically and clinically heterogeneous disorder and can be divided into early- and late-onset forms. The early-onset form includes maturity-onset diabetes of the young (MODY), a genetically heterogeneous monogenic form of NIDDM characterized by early age of onset (usually <25 years) and autosomal dominant inheritance (3). Linkage studies have identified genes that are

mutated in different MODY pedigrees on chromosomes 20 (*MODY1* locus, hepatocyte nuclear factor-4 α [HNF-4 α] gene), 7 (*MODY2* locus, glucokinase gene), and 12 (*MODY3* locus, HNF-1 α gene) (4–6), and clinical studies indicate that mutations in these genes are associated with abnormal patterns of glucose-stimulated insulin secretion (7–10).

Although there has been considerable success in identifying the genes for MODY, there has been relatively little progress in identifying the genes responsible for the more common forms of late-onset NIDDM; they do not appear to have a simple Mendelian basis and are thought to result from the joint action of genetic and environmental factors. This lack of progress can be attributed to a number of factors, including the paucity of extended pedigrees because of the late age of onset of the disorder, the prevalence of bilineal inheritance, and the genetic heterogeneity that occurs within and between pedigrees (2). To optimize the likelihood of finding novel NIDDM susceptibility genes, we have chosen to study an extended pedigree in which late-onset NIDDM segregates in a pattern consistent with an autosomal dominant disorder. Our strategy was to undertake linkage analysis with the three loci that are known to cause MODY followed by a systematic search for linkage if those results were negative. We report positive linkage between markers at the *MODY3* region and NIDDM in this pedigree.

RESEARCH DESIGN AND METHODS

The protocol was approved by the Princess Alexandra Hospital Research Ethics Committee, and informed consent was obtained from all participants. The proband and relatives were clinically characterized by a diabetes physician or a clinical nurse, with the majority of the assessments performed in the homes of the study participants. The protocol included a standardized interview that recorded age of onset of diabetes and history of its treatment and complications; measurement of height, weight, BMI, and blood pressure; and collection of a fasting blood sample for measurement of glucose, insulin, and C-peptide levels. For deceased study subjects, information about diabetes (age of onset and type of treatment) was obtained from spouse or offspring and, where possible, confirmed by medical record review.

Plasma glucose was measured by a hexokinase method using the Hitachi 747–100 analyzer (Boehringer Mannheim, Mannheim, Germany) with CV <2%. Plasma C-peptide was measured by radioimmunoassay (RIA; Diagnostic Systems Laboratories, Webster, TX). Cross-reactivity with proinsulin species was negligible. The fasting plasma glucose and C-peptide concentrations were interpreted by homeostasis model assessment of β -cell function and insulin sensitivity (11). This method uses a mathematical model of the body's glucose and insulin interactions as a frame of reference. The major feedback loops are stimulation of insulin secretion by glucose, reduction of hepatic glucose output, and increase in uptake of glucose into muscles by insulin. Different degrees of insulin resistance and impaired β -cell function can be introduced into the model, and for each combination the homeostatic fasting plasma glucose and C-peptide results achieved by the feedback loops are calculated. Each patient's fasting plasma glucose and C-peptide measurements can be interpreted by the model to predict the β -cell function and insulin sensitivity that are likely to have given those meas-

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FPG, fasting plasma glucose; HNF, hepatocyte nuclear factor; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction; TBE, tris-borate-EDTA.

urements. β -Cell function and insulin sensitivity are expressed as centile groups defined relative to a nondiabetic population <35 years old and <115% of ideal weight. β -cell function and insulin sensitivity measured by homeostatic model assessment have been shown to correlate with measures obtained by hyperglycemic and euglycemic clamp (11,12).

The following subjects were considered affected: 1) those with diabetes diagnosed according to World Health Organization criteria and treated with oral hypoglycemic agents, insulin, or a specific diet and 2) those with impaired fasting glucose as defined by the American Diabetes Association (fasting plasma glucose [FPG] = 6.1 mmol/l) (13). Normoglycemia was defined as FPG = 5.5 mmol/l.

DNA was extracted from blood samples using the salting-out method (14). Paternity was established by the use of highly polymorphic markers in a protocol based on polymerase chain reaction (PCR) and the incorporation of [³²P]dCTP during amplification. Based on the Cooperative Human Linkage Centre database and the physical map, we selected four markers that span the expanded *MODY3* region: D12S86, D12S321, D12S807, and D12S342. PCR reactions were performed in 96-well plates in 10- μ l volumes containing 50 ng genomic DNA, 1 \times PCR buffer (Perkin Elmer Cetus, Norwalk, CT), 0.5 pmol of each primer, 0.5 U of AmpliTaq Gold (Perkin Elmer Cetus), 2.5 mmol/l MgCl₂, 1 μ Ci [³²P]dCTP (Bresatec, Adelaide, South Australia), 0.025 mmol/l dCTP, and 0.25 mmol/l each of dATP, dGTP, and dTTP (Pharmacia Biotech, Uppsala, Sweden). After a 10-min initial denaturing step at 94°C in a 96-well thermal cycler (Corbett Research, Sydney, Australia), samples were subjected to 40 cycles of 15 s at 55°C for annealing, 72°C for 30 s, and 94°C for 15 s for denaturing; a final 5-min incubation took place at 72°C. After amplification, the products were denatured by the addition of 9 μ l sequencing stop solution followed by heating at 95°C for 5 min. Next, 2 μ l of each sample was loaded onto a 6% denaturing polyacrylamide gel in 1 \times TBE (tris-borate-EDTA) buffer and resolved electrophoretically at 100–120 W. Gels were fixed, dried, and exposed to autoradiographic film. After film development, microsatellite genotypes were scored without knowledge of the clinical or pedigree data. Consistency within and between gels was maintained by using size standards.

Linkage analysis was performed using the FASTLINK 2.0 versions of MLINK and LINKMAP (15–17), which performed multipoint analyses at each location along chromosome 12q spanned by the region D12S86, D12S321, D12S807, and D12S342. Four liability classes were defined on the basis of age: class 1, >60 years; class 2, 40–60 years; class 3, 20–40 years; and class 4, <20 years. To accommodate for poten-

tial heterogeneity, phenocopy rates were used for each liability class (0.01, 0.001, 0.0001, and 0.0001, respectively) (18). The penetrance factors used for the heterozygous dominant genotype were 0.90, 0.75, 0.45, and 0.15, and for the homozygous dominant genotype, 0.95, 0.90, 0.90, and 0.80. The frequency of the disease allele was estimated to be 5%. The allele frequencies were calculated from the pedigree data.

Using primers described previously (19), the 10 exons, the flanking intronic sequence, and the promoter region were amplified by PCR. Ten members of the pedigree were included in the sequencing studies, and both DNA strands were sequenced. Another pair of primers, BGF (5'-AGC CAG CAC TGT TCT TGG CAC-3') and BGR (5'-GAC TTC AGC CCT GCA AAG TGC AGG-3'), was designed to generate a 430-bp fragment further upstream of, and partially overlapping, the most 5' region amplified by Kaisaki et al. (19). The PCR products were sequenced directly using an ABI Prism Big Dye automated sequencing kit and an ABI Prism 377 sequencer. Sequence data was compared with that previously presented (19).

RESULTS

The pedigree structure and *MODY3* haplotypes at markers D12S86, D12S321, D12S807, and D12S342 are shown in Fig. 1, along with inferred haplotypes for the deceased founders. The haplotype assignments were confirmed with the GENEHUNTER program (20). The haplotype 5654 was found to segregate with diabetes in the pedigree. Individual IV-6 is an obligate recombinant, placing the NIDDM locus telomeric of marker D12S321. Subjects II-7 and III-20 represent possible phenocopies. Subjects IV-1 and IV-3 have inherited the at-risk haplotype but are nonpenetrant at this stage. Because of the strong family history of NIDDM, these two subjects adopted a low-fat diet and a physical activity program; their BMIs are 26 and 20 kg/m². Subject IV-5 was diagnosed with insulin-requiring diabetes at age 8 years and had a clinical history consistent with IDDM.

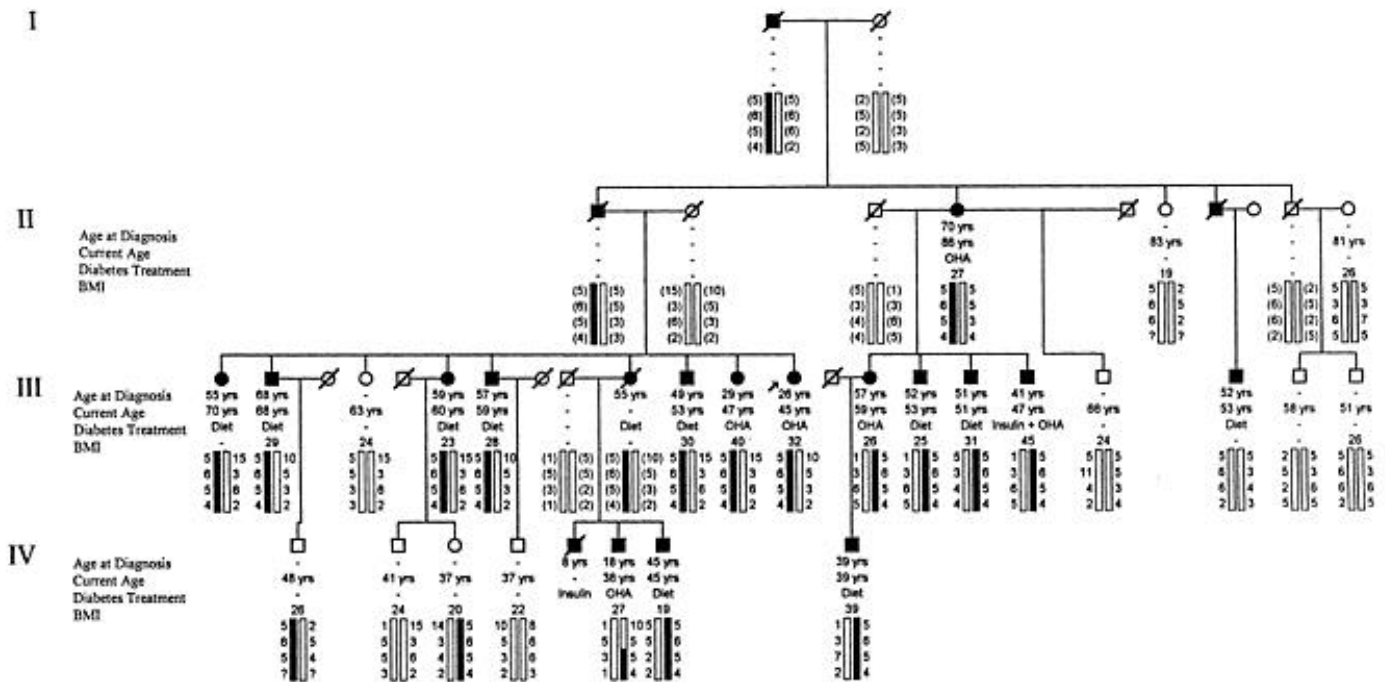


FIG. 1. NIDDM pedigree (● ■, affected family members; ○ □, normoglycemic subjects; ✕, proband; /, deceased subjects). The clinical features of the subjects are noted below each symbol, including age at diagnosis, present age, diabetes treatment (diet, oral hypoglycemic agent [OHA], and insulin therapy), and BMI expressed as weight in kilograms divided by height in meters squared. The genotypes at markers D12S86, D12S321, D12S807, and D12S342 are shown, and the haplotypes based on GENEHUNTER estimates are indicated. The haplotypes for deceased founders and other unavailable individuals are inferred and indicated within parentheses. The at-risk haplotype is noted by shading. Individual IV-6 is an obligate recombinant, placing the NIDDM locus telomeric of marker D12S321. Subjects II-7 and III-20 represent possible phenocopies. Subjects IV-1 and IV-3 have inherited the at-risk haplotype but are nonpenetrant at this stage.

Age at diagnosis of NIDDM for those affected members of the pedigree available for testing was 48 ± 14 years (mean \pm SD), and BMI was 30 ± 7 kg/m². Eleven subjects required treatment with diet alone, five were on oral hypoglycemic therapy, and one was on nocturnal long-acting insulin in combination with sulfonylurea. Eight of the diabetic subjects had clinical evidence of macrovascular complications (ischemic heart disease or cerebrovascular disease). Subject IV-6, who had had NIDDM for 20 years, had diabetic nephropathy and proliferative retinopathy. The affected subjects had heterogeneous β -cell function (median 92%, interquartile range 47–140%) and substantial insulin resistance (median 35%, interquartile range 16–42%) relative to a normal reference value of 100%.

Two-point linkage analysis logarithm of odds (LOD) scores for each of the *MODY3* markers with NIDDM in the pedigree are presented in Table 1. A graphic representation of the results of parametric multipoint linkage analysis of the markers at the *MODY3* locus are shown in Fig. 2. The maximal LOD score was 3.65 at $\theta = 0.008$ telomeric to marker D12S321.

For 10 pedigree members, we sequenced the 10 exons and the promoter of the HNF-1 α gene in both directions and found no evidence of a mutation coinheritance with NIDDM in the pedigree, although several previously described polymorphisms were found in affected and unaffected individuals (19). No mutations or polymorphisms were found in the minimal promoter of the HNF-1 α gene, a region containing the binding sites for HNF-4 α and HNF-3, both of which have been implicated in the regulation of HNF-1 α expression (19). No variants were identified in exons 2, 3, 4, 5, 6, 8, 9, or 10 of HNF-1 α . Three previously described polymorphisms in exon 1 were identified in affected and unaffected pedigree members: a C-G Leu/Leu polymorphism in codon 17, an A-C Ile/Leu polymorphism in codon 27, and a C-T Ala/Val polymorphism in codon 98. Two previously described polymorphisms were identified in affected and unaffected pedigree members in exon 7: a C-T Leu/Leu polymorphism in codon 459 and a G-A Ser/Asn polymorphism in codon 487 (19).

DISCUSSION

This report of a large pedigree with late-onset NIDDM associated with insulin resistance presents evidence of linkage between late-onset NIDDM and markers in the *MODY3* region of chromosome 12q. There was no evidence of mutation in the HNF-1 α gene or its promoter, and we hypothesize that another gene or genes in this region of chromosome 12q contribute to diabetes susceptibility in the pedigree.

Our negative mutation data for HNF-1 α is not surprising given the phenotype of affected subjects in our pedigree. Sub-

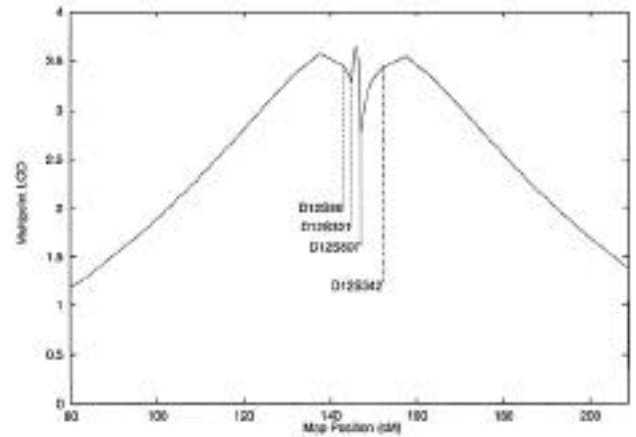


FIG. 2. Multipoint location score curve for *MODY3* with the near-dominant model and markers D12S86, D12S321, D12S807, and D12S342.

jects with mutations in HNF-1 α are described as having diabetes characterized by an early age at diagnosis, predominant insulin deficiency, lean body mass, early microvascular complications, and an early therapeutic requirement for insulin (10,21). In contrast, the affected subjects in our pedigree were obese, had a relatively late age at diagnosis, were often treated with diet, had a low prevalence of microvascular complications but a high prevalence of macrovascular disease, and pathophysiologically had predominant insulin resistance associated with heterogeneous levels of β -cell function.

That there is genetic heterogeneity in diabetes susceptibility between different ethnic groups would appear evident from other reports concerning the role of the *MODY3* locus in NIDDM. There is no evidence of linkage of NIDDM with this locus in Mexican-American (22) or Pima Indian sib pairs (23,24). Lesage et al. (25) reported no evidence for linkage of NIDDM with *MODY3* markers in a large collection of French NIDDM families. Mahtani et al. (26) described linkage of *MODY3*-linked markers with NIDDM in a subset of families from an isolated population in western Finland, the linkage being present in the group with the lowest insulin secretion. The authors inferred the presence of a gene in this region ("*NIDDM2*") that affects susceptibility to adult-onset diabetes associated with low insulin secretion. The Finnish families in the low-insulin quartile had a later age of onset (mean 58 years) than that described for families with HNF-1 α mutations. In the current study, the pathophysiological defect in the affected pedigree members is predominantly insulin resistance with heterogeneous levels of β -cell function. Nevertheless, it is possible that the locus identified by Mahtani et al. (26) corresponds with the one identified in the present study, as impaired β -cell compensation in the context of insulin resistance is an important factor in the development of hyperglycemia (1). Bowden et al. (27) documented evidence for linkage of NIDDM with markers in the *MODY3* region in Caucasian sib pairs with a history of adult-onset nephropathy but did not demonstrate linkage in African-American sib pairs. Ours is the first report of late-onset NIDDM associated with severe insulin resistance showing linkage to the *MODY3* region. The ethnic background of the pedigree is relevant because the founder was a Pacific Islander, and NIDDM in

TABLE 1
Two-point linkage analysis LOD scores for the *MODY3* markers versus affection status in the pedigree

| Markers | Recombination fraction (θ) | | | | | |
|---------|-------------------------------------|------|------|------|------|------|
| | 0 | 0.01 | 0.05 | 0.1 | 0.2 | 0.3 |
| D12S86 | 2.37 | 2.47 | 2.52 | 2.34 | 1.73 | 1.01 |
| D12S321 | 0.02 | 0.05 | 0.14 | 0.19 | 0.16 | 0.07 |
| D12S807 | 1.47 | 1.64 | 1.83 | 1.79 | 1.43 | 0.91 |
| D12S342 | 0.68 | 0.72 | 0.79 | 0.80 | 0.67 | 0.45 |

Oceania is commonly associated with severe insulin resistance. There are no other reports of linkage or mutation analysis with the *MODY3* locus in this population.

NIDDM is characterized by defects in both insulin secretion and insulin action. Hyperinsulinemia compensates for insulin resistance in the early prediabetic state, and the subsequent development of hyperglycemia results from the failure of β -cells to secrete enough insulin for effective compensation (1). The genetic susceptibility to NIDDM in the pedigree may affect β -cell function, insulin resistance, or both. Secondary defects due to hyperglycemia may be relevant to the combination of pathophysiologies in the affected family members. Alternatively, a single molecular abnormality may underlie both insulin resistance and the failure of effective β -cell compensation, as occurs with the disruption of *IRS2* in mice (28).

In summary, the results of our study indicate that the region of chromosome 12q close to *MODY3* harbors a novel susceptibility gene for late-onset NIDDM. Positional cloning will be undertaken to further refine its localization and define its nature in the pedigree.

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