Diagnostic Heterogeneity of Diabetes in Lean Young Adults

Classification Based on Immunological and Genetic Parameters

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The aim of our study was to investigate the relative prevalence of the different forms of diabetes in young adults and their respective clinical characteristics. Included were 51 nonobese patients (BMI < 27 kg/m^2) with diabetes diagnosed before age 40, excluding typical IDDM. Each patient was subjected to screening for glucokinase gene (MODY2) and mitochondrial DNA (at nucleotide 3243) mutations, to HLA class II genotyping, and screening for the presence of islet cell antibodies (ICAs) and anti-GAD antibodies. Informative families were analyzed for linkage of diabetes to chromosome 12q (MODY3). Based on clinical criteria, patients were subdivided into MODY (n = 19) and non-MODY (n = 32). In the MODY group, we identified three patients with MODY2, one with the 3243 mitochondrial mutation, and another with autoimmune diabetes. One of the five MODY families available for linkage study was shown to have MODY3. In the non-MODY group, we found five patients with autoimmune diabetes and one with MODY2. No clinical parameter was helpful to classify patients in one of these subclasses of diabetes; however, the glucagon-stimulated C-peptide was useful to discriminate between MODY2 patients and the others. In conclusion, young and lean non-insulin-dependent diabetic patients constitute a very heterogeneous group, although they present similar clinical characteristics. The clinical distinction of MODY and non-MODY patients allows correct classification in, at most, 75% of the patients and thus is not sufficient to predict clinical course. However, immunological and genetic parameters allowed us to classify only 25% of the patients in specific diagnostic classes. *Diabetes* 46:622–631, 1997

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FPG, fasting plasma glucose; ICA, islet cell antibody; IGT, impaired glucose tolerance; JDF U, Juvenile Diabetes Foundation unit; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

The World Health Organization defines two major types of diabetes mellitus: type I or insulin-dependent (IDDM) and type II or non-insulin-dependent diabetes mellitus (NIDDM). The two diseases are distinct with respect to etiology, pathogenesis, clinical course, and treatment. However, at diagnosis, discrimination between IDDM and NIDDM can be difficult, particularly in young adults (1).

Although IDDM is classically characterized by an abrupt onset of severe symptoms, absolute dependence on exogenous insulin, and proneness to ketosis, some patients may present with mild or no symptoms and no absolute need for insulin treatment (2,3). This slow clinical onset with a period of insulin independence may reflect a progressive loss of β-cells, the rate of which seems inversely related to age (4–7). Immunogenetic analyses based on the detection of islet cell antibodies (ICAs) and anti-glutamic acid decarboxylase (GAD) antibodies and HLA class II genotyping are important to identify patients with this form of autoimmune diabetes (8,9).

Other subgroups of young diabetic patients with no absolute requirement for insulin treatment belong to the NIDDM and maturity-onset diabetes of the young (MODY) types. True NIDDM in the young is poorly defined and probably rare, but its prevalence increases rapidly after 30 years of age (1). Young NIDDM patients are less frequently obese than are those with late-onset NIDDM, and a family history of diabetes is often present (10). A separate form of NIDDM in the young, called MODY, is defined by diagnosis before 25 years of age, transmission as an autosomal dominant trait, and no insulin treatment for at least 2 years after the clinical onset of the disease (11–14). It is now recognized that MODY is genetically heterogeneous and that at least four susceptibility genes are involved in the pathogenesis of this form of NIDDM.

The primary purpose of the study was to investigate the relative prevalence of the different forms of diabetes in lean young adults and their respective clinical characteristics. To this end, a group of nonobese patients with diabetes diagnosed before age 40, excluding typical IDDM, was studied. Based on genetic and immunological analyses, only 25% of the patients could be characterized. The possibility of differentiating these classes on the basis of conventional clinical and biological data was investigated.

RESEARCH DESIGN AND METHODS

Study design. Patients were recruited through a campaign of information directed toward patients (by advertising in a local diabetes journal) and physicians (through letters to the members of the Swiss Diabetic Association and individual physicians and through a collaborative effort between the five University Hospitals in Switzerland). Patients were selected with the following inclusion criteria: *1*) fasting plasma glucose >6.1 mmol/l and 2-h postprandial glycemia >7.8 mmol/l; *2*) age at diagnosis <40 years; *3*) leanness (BMI <27 kg/m^2); and *4*) Caucasian origin. Patients with classic IDDM presentation (abrupt onset of the disease, ketosis proneness, and insulin dependency), hyperglycemia secondary to endocrinopathies or pancreatic disease, or gestational diabetes (limited to the duration of pregnancy) were excluded. Clinical data were obtained for each diabetic patient during a standardized clinical examination by one of us (P.D.) or the patient's personal physician. Information about the onset of the disease as well as a detailed family history were collected. Based on these data, patients were tentatively assigned to a MODY or a non-MODY subgroup. Patients were investigated for secondary complications (both micro- and macroangiopathic). When an abnormal genetic pattern was found in the proband (see below), co-segregation analysis for the remaining available members of the family was done. Relatives were first screened by fasting plasma glucose (FPG), and in cases of borderline values (FPG >5.5 mmol/l), an oral glucose tolerance test (OGTT) was performed. They were considered affected if they fulfilled criteria for impaired glucose tolerance or diabetes using the World Health Organization definition or if they were treated for diabetes. The study protocol was approved by the ethical committee of the Department of Medicine of the University Hospital of Geneva, and informed consent was obtained from each patient.

Biological investigations. Fasting blood glucose was determined by the glucose oxydase method. HbA_{1c} was determined by the DCA 2000 analyzer (Miles, Elkhart, IN). The plasma C-peptide level was measured by radioimmunoassay (Biodata, Ares Serono Diagnostic, or the Diagnostic Product Corporation kit) after an overnight fast (basal C-peptide, determined in 97% of the patients) and 6 min after the intravenous injection of 1 mg of glucagon (stimulated C-peptide, in 88% of the patients) to evaluate insulin secretory reserve. Reference values were identical for both tests (basal levels of 0.3 to 0.9 nmol/l for fasting and resting subjects). In about one-third of the patients, the test was done twice and the mean value used for statistical analyses. The increment of C-peptide was defined by calculating the difference between stimulated and basal levels expressed as percentage of basal level. Cytoplasmic ICA and anti-GAD antibodies in sera were determined by indirect immunofluorescence (15) and by radioimmunoassay, respectively (16). The anti-GAD assay performed by Jean-Claude Ongagna in the laboratory of Claire Levy-Marchal (Hôpital Robert Debré, Paris, France) had a sensitivity of 78% and a specificity of 98% as demonstrated in the second Immunology of Diabetes Workshop (1995).

Genetic analysis. DNA was extracted from peripheral blood samples using a rapid and nonenzymatic method (17). The 12 exons of the glucokinase gene were scanned for mutations using polymerase chain reaction (PCR) amplification followed by analysis of single-strand conformation polymorphism (SSCP) on a minigel system (PhastSystem, Pharmacia, Uppsala, Sweden), as described elsewhere (18,19). Amplification products showing abnormal electrophoresis migration patterns were subjected to direct sequencing using a cycling procedure (Cyclist, Stratagene, La Jolla, CA). Digestion of PCR products of exon 6 by *Hph* I was used to detect the T→C mutation at codon 203 (V203A) in families of probands carrying this mutation. The A→G transition mutation at nucleotide 3243 of the mitochondrial DNA (tRNA $^{Leu(UUR)}$) was detected by PCR followed by *Apa* I enzymatic digestion and electrophoresis using the PhastSystem (20).

For linkage study to chromosome 12q, simple sequence repeat polymorphic markers were typed using a PCR-based method. All oligonucleotide sequences for the amplification of AFM123xh2 (D12S86), AFM010th7 (D12S76), AFMa114xf1 (D12S1349), and AFM294ze9 (D12S342) microsatellite markers are available in the Genome Data Base. Three additional markers derived from the poly(CA) microsatellites Genethon map have been tested within the D12S86–D12S342 interval: D12S1721 (AFMa082za5), D12S1603 (AFMa189xe1), and D12S1611 (AFMa204za5) (21). PCR reactions and electrophoresis were performed as previously described (22).

Two point linkage analyses were performed with the LINKAGE programs (23). The genetic data were analyzed using a previously described model (24) with a frequency for the disease allele of 0.001, and equal female-to-male recombination rates were assumed. Because of the incomplete penetrance of MODY and its age-dependent expression, logarithm of odds (LOD) scores were calculated with four age-dependent liability classes: <10, 10–25, 25–40, and >40 years.

HLA class II genotyping was performed by hybridization with sequence-specific oligonucleotide probes after exon 2 PCR amplification of DRB1/DRB3/DRB4/DRB5 and DQB1 loci (25).

Statistical analysis. Statistical comparisons were done by contingency-table χ^2 test for qualitative traits and by t test or Mann-Whitney test for parametric or nonparametric quantitative traits, respectively. A P value of 0.05 or less was considered statistically significant.

RESULTS

Characteristics of the whole population. During an inclusion period of 2 years, 51 unrelated young and lean patients with impaired glucose tolerance (IGT) (n = 3) or diabetes (n = 48) aged 18 to 55 (mean 33.3 ± 7.6) years were recruited. Sex distribution was equal (Table 1), and mean age at diagnosis was 24.8 ± 7.6 years (ranging from 13 to 39). Diagnosis was made incidentally in half of the cases; patients with IGT were detected after a random glucose measurement in subjects with a family history of diabetes. Symptoms of polyuria and polydipsia were present in only a third of patients. In six women, hyperglycemia was first discovered during pregnancy. Twenty-one patients (41%) were treated with insulin at the time of study, but none were insulin dependent (ketosis prone); this treatment was initiated in the majority of cases in the 1st year following diagnosis (67%). A positive family history was found in 37 patients (73%), and the mode of inheritance was compatible with an autosomal dominant transmission (at least two generations, one or more first-degree relatives affected) in 35 patients. For two patients, both parents had diabetes. Chronic diabetic complications were found in four subjects. Neuropathy and retinopathy were each detected in two patients. Nephropathy or macrovascular complication were not found.

Clinical and biological characteristics of clinically defined MODY patients. Subjects were classified clinically as MODY and non-MODY patients. Clinical criteria used to define MODY patients were diabetes diagnosed before 25 years of age, apparent disease transmission as an autosomal dominant trait, and treatment for at least 2 years without insulin. Nineteen patients (37%) fulfilled these criteria. They differed from non-MODY patients by the mean duration of diabetes at the time of study (15.1 ± 10.8 vs. 4.6 ± 5.3 years, P < 0.001). Of the 37 subjects with positive family history, 19 were included in the clinical MODY group and 18 in the non-MODY group; of these patients, 18/19 in the MODY group (95%) and 15/18 (83%) in the non-MODY group had only one or two affected relatives. Compared with the MODY group, more patients in the non-MODY group had the HLA DQB1*0302 allele (22 vs. 0%, P = 0.03) or a nonaspartic amino acid in position 57 of the DQ allele (59 vs. 32%, P = 0.05), genotypes commonly associated with IDDM (26). The frequency of diabetic complications was similar in the two groups (Table 1).

Patients with a glucokinase gene mutation. By screening all exons of the glucokinase gene, we found three sequence abnormalities. A G→A transition located eight base pairs upstream from the beginning of exon 3 was detected in two unrelated patients. Analysis of the glucokinase messenger RNA from leukocytes by reverse transcription followed by PCR amplification of exons 2–5 did not result in the appearance of any abnormal length products (data not shown), suggesting that this sequence variation does not affect splicing of the primary transcript. Another intronic variation (T→C, 17 base pairs upstream from the beginning of exon 4) was found in one subject who had no family history of diabetes. These two mutations are likely to represent

TABLE 1
Comparison of probands classified with clinical and pathophysiological parameters

Characteristics	Whole population	MODY patients	Non-MODY patients	MODY2 patients	ICA$^+$ patients
n	51	19	32	4	6
Sex (M/F)	21 (41)/30 (59)	8 (42)/11 (58)	13 (41)/19 (59)	3 (75)/1 (25)	3 (50)/3 (50)
Age (years)	33.3 ± 7.6	33.8 ± 8.9	33.0 ± 6.8	36.5 ± 9.4	31.5 ± 5.3
BMI (kg/m^2)	22.4 ± 2.5	22.4 ± 2.9	22.3 ± 2.3	23.3 ± 2.0	22.3 ± 2.8
Age at diagnosis (years)	24.8 ± 7.6	18.6 ± 4.1*	28.5 ± 6.8	21.7 ± 11.1	29.2 ± 3.7
Duration of diabetes (years)	8.5 ± 9.2	15.1 ± 10.8*	4.6 ± 5.3	14.7 ± 10.8‡	2.5 ± 2.5
Presentation					
Incidental	25 (49)	13 (69)†	12 (36)	3 (75)	2 (33.3)
Symptoms	17 (33)	4 (21)	13 (41)	0	2 (33.3)
Pregnancy	6 (12)	1 (5)	5 (17)	0	2 (33.3)
Other	3 (6)	1 (5)	2 (6)	1 (25)	0
Fasting glycemia (mmol/l)	8.0 ± 2.9	7.9 ± 2.8	8.0 ± 3.0	8.2 ± 0.9	7.40 ± 2.35
HbA_{1c} (%)	7.13 ± 1.55	6.8 ± 1.49	7.33 ± 1.58	6.67 ± 1.02	7.43 ± 1.73
Basal C-peptide (nmol/l)	0.501 ± 0.270	0.539 ± 0.274	0.477 ± 0.269	0.743 ± 0.248	0.397 ± 0.164
Stimulated C-peptide (nmol/l)	0.992 ± 0.580	1.088 ± 0.594	0.935 ± 0.575	1.774 ± 0.564‡	0.705 ± 0.333
Increment of C-peptide (%)	105 ± 61	109 ± 58	103 ± 63	143 ± 34‡	82 ± 36
Treatment					
Diet alone	18 (35)	8 (42)	10 (31)	4 (100)	2 (33)
Oral agent	12 (24)	7 (37)	5 (16)	0	1 (17)
Insulin	21 (41)	4 (21)†	19 (53)	0	3 (50)
Complications	4 (8)	2 (10)	2 (6)	0	0
Positive family history	37 (73)	19 (100)*	18 (56)	4 (100)	4 (67)
Glucokinase mutation	4 (8)	3 (16)	1 (3)	4 (100)	0
ADNmit(3243) mutation	1 (2)	1 (5)	0	0	0
HLA DR3 and/or 4	21 (41)	6 (32)	15 (47)	2 (50)	6 (100)
HLA DQB1*0302	7 (14)	0†	7 (22)	0	3 (50)
Non-Asp 57 of DQB1	25 (49)	6 (32)†	19 (59)	1 (25)	4 (67)
Anti-GAD	3 (7)	1/15 (7)	2/28 (7)	0	0
ICA >20 JDF U	6/48 (12.5)	1/17 (6)	5/31 (16)	0‡	6 (100)

Data are means ± SE or *n* (%). MODY is defined as diabetes transmitted as an autosomal dominant trait diagnosed before 25 years of age and treated for at least 2 years without insulin. MODY2, the four probands with the V203A glucokinase mutation; ICA$^+$, the six probands with ICA >20 JDF U. *P < 0.001, difference between MODY and non-MODY patients; †P < 0.05, difference between MODY and non-MODY patients; ‡P < 0.05, difference between MODY2 and ICA$^+$ patients.

represent polymorphisms with no physiological or clinical consequences.

Four unrelated subjects were found to be heterozygous carriers of a missense mutation (T→C) in codon 203 modifying the amino-acid sequence of the protein (valine to alanine, = V203A). This mutation was described in a previous study (27) and was shown to cause a dramatic decrease in enzyme activity (28). The mutation co-segregated with diabetes in the families of the four probands (Fig. 1). Age at diagnosis of the four subjects with the V203A glucokinase mutation (MODY2 patients) ranged from 14 to 38 (mean 21.7 ± 11.1) years (Table 1). No patients presented symptoms related to hyperglycemia. Metabolic control (mean HbA_{1c} level 6.7%) was achieved in the four patients with diet alone. MODY2 patients could be distinguished from other MODY or autoimmune diabetes (ICA$^+$) patients by a statistically higher glucagon-stimulated C-peptide level (P = 0.03 and 0.02, respectively); the increment of C-peptide was also higher compared with ICA$^+$ patients (P = 0.03). The prevalence of glucokinase mutation in the clinically defined MODY group was only 16%, but one patient with the glucokinase mutation was initially assigned to the non-MODY group because of diagnosis at 38 years of age (Table 1).

Patients with ICAs and anti-GAD antibodies. Of 48 subjects tested for ICA, 14 (29%) had detectable antibodies: five with <10 Juvenile Diabetes Foundation units (JDF U), three with 10–20 JDF U, and six (12.5%) with >20 JDF U. Taken as a whole group, the frequency of patients with the HLA-DR1 allele was increased to more than twofold in our diabetic population (33 vs. 14.5%) compared with a control group of 110 volunteer blood donors from Geneva. In the subset of patients with positive ICAs, the frequency of the class II HLA alleles commonly associated with IDDM, DRB1*03 and/or *04 and DQB1*0302, was increased compared with ICA-negative patients. However, we considered levels <20 JDF U to be of low specific value; thus only the six patients with ICA levels above this limit were considered affected by autoimmune diabetes. Diagnosis was made incidentally in two patients, during pregnancy in another two, and in the context of hyperglycemic symptoms in the last two subjects (Table 1). Mean age at diagnosis was 29.2 ± 3.7 (range 25–35) years, corresponding to the 75th percentile of the whole population. None of the subjects was insulin-dependent at the time of study, but three were receiving insulin therapy. Two patients were well controlled with diet alone and one with oral agents. Basal and stimulated C-

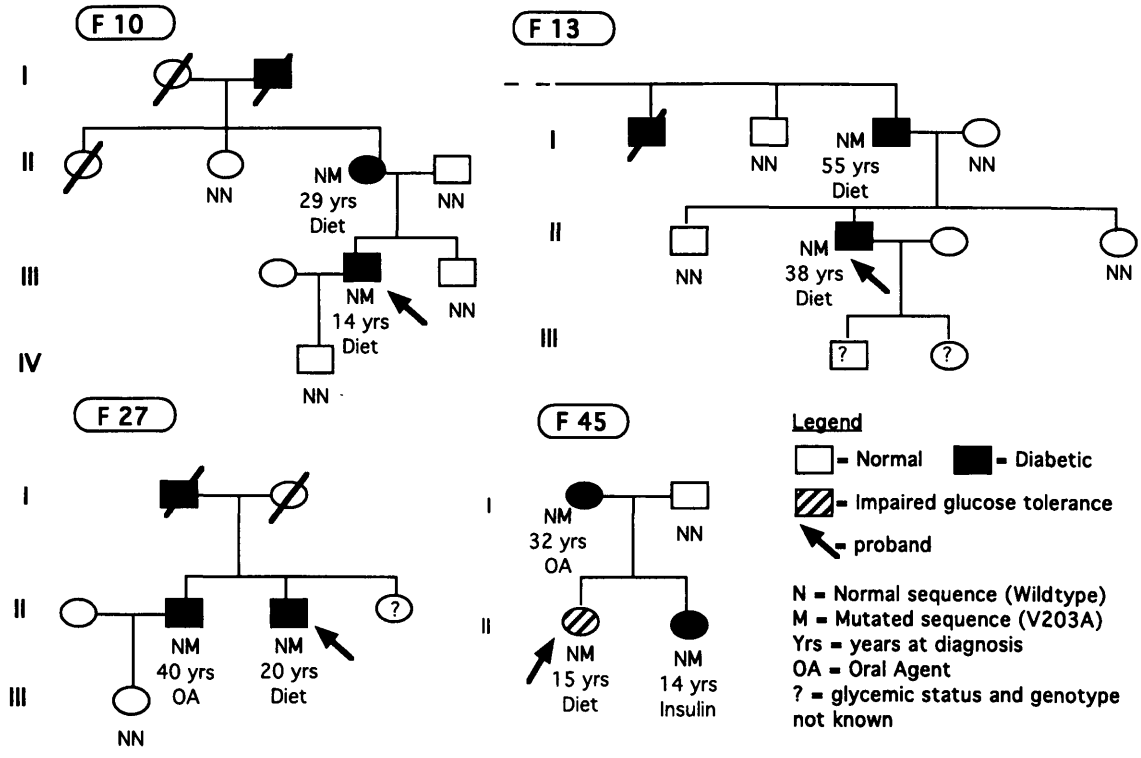

FIG. 1. Pedigrees of the four unrelated probands with the V203A mutation of the glucokinase gene.

peptide levels (0.397 ± 0.164 and 0.705 ± 0.333 nmol/l, respectively) were in the low normal range, indicating a relatively preserved insulin secretory reserve. Whereas five subjects were correctly included in the non-MODY group, one patient fulfilled clinical criteria for MODY.

Anti-GAD antibodies were detected in only three subjects with a duration of diabetes ranging from 2 to 42 years. None of these patients was insulin treated and none had detectable ICA; one patient was included in the MODY group and the other two in the non-MODY group.

Patients with the 3243 mitochondrial DNA mutation. The 3243 (tRNA $^{Leu(UUR)}$) mitochondrial DNA mutation was detected in one patient. The family history of this subject was compatible with maternal transmission. Diabetes was diagnosed at age 24 with typical symptoms; insulin was needed only transiently during an acute decompensation 3 years later. The patient did not complain of hearing loss. Other family members were not available for co-segregation analysis. Based on clinical features initially available, this subject was originally included in the MODY group.

Linkage to the chromosome 12q. Linkage analysis using microsatellite markers of the chromosome 12q region harboring the putative MODY3 gene (22) was performed for five MODY probands not affected by a glucokinase mutation and their families (Fig. 2). The results of the LOD score calculation are shown by family in Table 2 for the seven markers tested. One family (F4) presents a positive LOD score value at θ (max) = 0.00 for all the markers, and the maximum LOD score (Z = 1.01) is obtained for the D12S76 locus. The four other families present negative individual LOD score values (< −1.70) at θ = 0.00 for two different markers of the D12S86–D12S342 interval. Figure 3 shows the pedigree of family F4 with the haplotype of the seven markers: all six affected individuals with MODY exhibited a common haplotype [12-2-10-6-5-2-2], also shared by an unaffected 24-year-old individual. The young unaffected child of patient 7 shows three distinct recombination events among the alleles making up the susceptibility haplotype. In family F4, these results are compatible with linkage of diabetes with the chromosome 12q region.

Clinical and biological features in genetically distinct forms of MODY. Clinical characteristics were compared in families of the three genetic subgroups of MODY patients distinguishable in the present study, namely those with a glucokinase mutation (MODY2), those with diabetes linked to the chromosome 12q marker (MODY3), and those not belonging to either of these two subgroups (MODYx) (Table 3). Metabolic control was less satisfactory in the MODY3 family compared with the MODY2 families as demonstrated by higher levels of fasting blood glucose (10.3 ± 2.7 vs. 7.34 ± 1.48 mmol/l, P = 0.02) and HbA_{1c} (7.8 ± 0.1 vs. 6.34 ± 0.8%, P = 0.01), although mean age and duration of diabetes were similar or even lower for MODY3 subjects. Diabetic patients of MODY2 families were more frequently treated with diet alone compared with MODYx patients (P = 0.001) and tended to have fewer chronic diabetic complications (P = 0.03). Whereas basal C-peptide levels did not differ between these three groups, glucagon-stimulated C-peptide levels were higher in MODY2 compared with MODY3 or MODYx patients (P = 0.04 and 0.004, respectively). In addition, the C-peptide increment was significantly higher in MODY2 than in MODYx patients (P = 0.005). BMI and fasting glycemia determined at the time of C-peptide measurement were similar in these groups and therefore could not explain the difference observed for the glucagon-stimulated C-peptide.

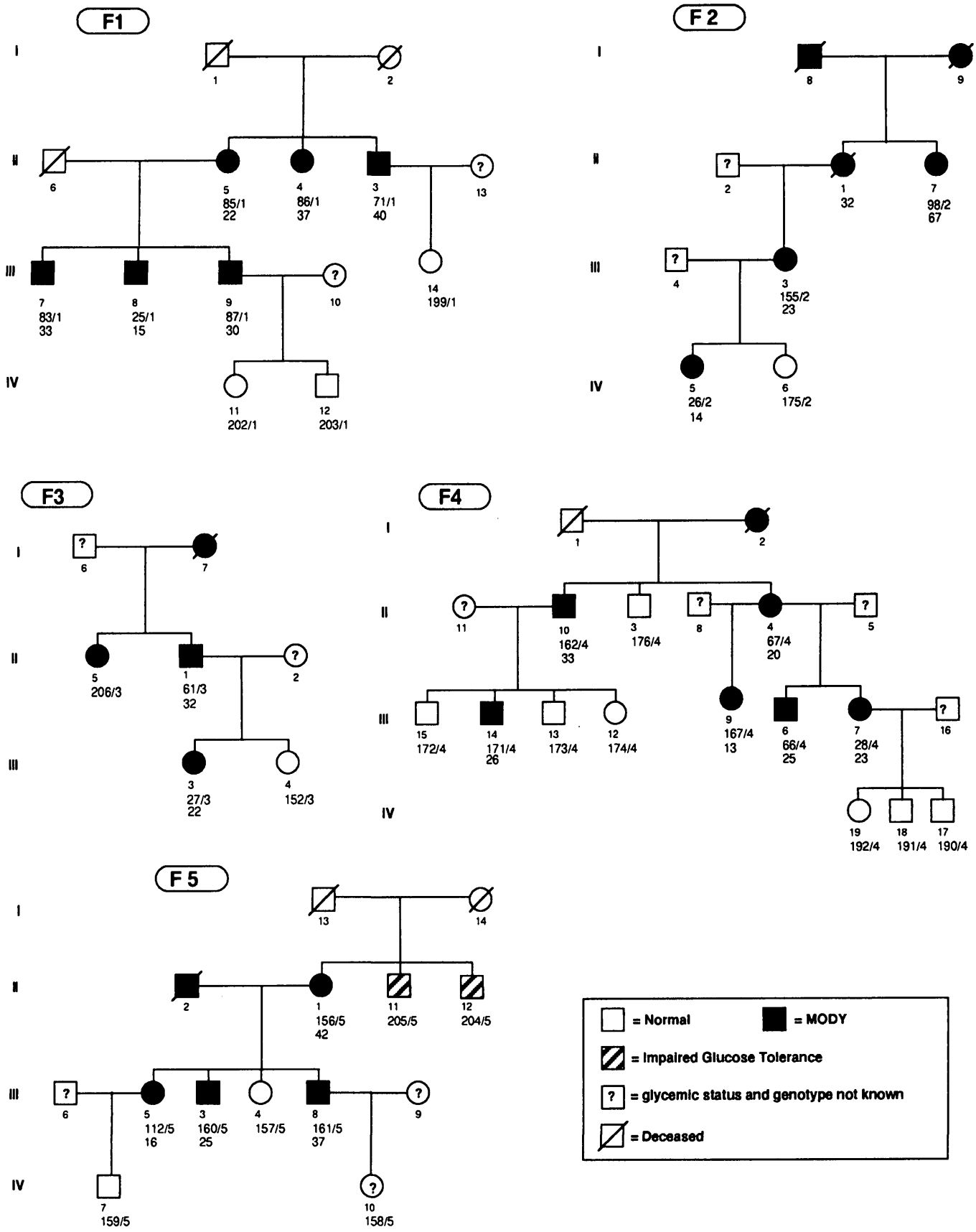

FIG. 2. Pedigrees of the five MODY families studied for linkage with the chromosome 12q marker. Numbers under the symbols correspond to the identification numbers. The age at diagnosis and status of each subject are shown. Only the F4 family presents a positive LOD score for the 12q locus.

TABLE 2
Pairwise LOD scores (Z) between microsatellite markers of chromosome 12q region and MODY phenotype in five families

	Recombination fraction θ							
	0.00	0.02	0.04	0.06	0.08	0.10	0.12	0.20
D12S86 (AFM123xh2)								
Total LOD score (θ = 0.02)	−7.31	−3.08	−2.03	−1.41	−1.01	−0.71	−0.51	−0.05
F1	0.32	0.30	0.28	0.26	0.24	0.21	0.19	0.12
F2	−1.98	−0.75	−0.50	−0.35	−0.26	−0.19	−0.14	−0.04
F3	−2.04	−0.90	−0.64	−0.49	−0.38	−0.30	−0.25	−0.10
F4	0.96	0.92	0.88	0.84	0.79	0.75	0.70	0.50
F5	−4.57	−2.65	−2.05	−1.67	−1.40	−1.18	−1.01	−0.53
D12S1721 (AFMa082za5)								
Total LOD score (θ = 0.00)	−1.23	−0.20	0.02	−0.14	0.19	0.25	0.28	0.30
F1	−0.44	−0.46	−0.45	−0.43	−0.41	−0.37	−0.34	−0.20
F2	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.00
F3	−2.04	−0.92	−0.65	−0.50	−0.40	−0.32	−0.26	−0.11
F4	0.94	0.90	0.86	0.82	0.77	0.72	0.68	0.48
F5	0.29	0.27	0.25	0.24	0.22	0.21	0.19	0.13
D12S76 (AFM010th7)								
Total LOD score (θ = 0.03)	0.75	1.49	1.61	1.65	1.62	1.57	1.50	1.17
F1	−1.50	−0.67	−0.44	−0.30	−0.22	−0.16	−0.11	−0.02
F2	0.40	0.38	0.36	0.34	0.32	0.30	0.28	0.21
F3	0.07	0.07	0.06	0.06	0.05	0.05	0.04	0.03
F4	1.01	0.97	0.92	0.88	0.83	0.78	0.73	0.53
F5	0.77	0.74	0.71	0.67	0.64	0.60	0.56	0.42
D12S1349 (AFMa114xf1)								
Total LOD score (θ = 0.01)	−1.97	−0.74	−0.31	−0.01	0.19	0.33	0.43	0.54
F1	−1.46	−1.32	−1.08	−0.87	−0.70	−0.56	−0.45	−0.18
F2	0.69	0.66	0.63	0.60	0.56	0.53	0.50	0.38
F3	−2.04	−0.89	−0.63	−0.48	−0.37	−0.30	−0.24	−0.10
F4	0.96	0.92	0.88	0.84	0.79	0.75	0.70	0.50
F5	−0.12	−0.11	−0.11	−0.10	−0.09	−0.09	−0.08	−0.06
D12S1603 (AFMa189xe1)								
Total LOD score (θ = 0.00)	−5.21	−2.78	−1.94	−1.39	−1.02	0.75	0.54	0.11
F1	−1.93	−1.61	−1.26	−1.00	−0.80	−0.65	−0.53	−0.23
F2	−1.96	−0.88	−0.63	−0.48	−0.39	−0.32	−0.26	−0.14
F3	−0.20	−0.18	−0.16	−0.14	−0.13	−0.11	−0.10	−0.06
F4	1.00	0.96	0.91	0.87	0.82	0.77	0.72	0.51
F5	−2.12	−1.07	−0.80	−0.64	−0.52	−0.44	−0.37	−0.19
D12S1611 (AFMa204za5)								
Total LOD score (θ = 0.05)	−6.86	−3.23	−2.15	−1.48	−1.03	−0.71	−0.47	0.03
F1	1.74	−1.47	−1.14	−0.89	−0.70	−0.56	−0.44	−0.17
F2	−1.98	−0.75	−0.50	−0.35	−0.26	−0.19	−0.14	−0.04
F3	−2.04	−0.92	−0.65	−0.50	−0.39	−0.32	−0.26	−0.11
F4	0.95	0.91	0.87	0.83	0.78	0.74	0.69	0.50
F5	−2.05	−1.00	−0.73	−0.57	−0.46	−0.38	−0.32	−0.15
D12S342 (AFM294ze9)								
Total LOD score (θ = 0.05)	−1.08	0.11	0.33	0.42	0.47	0.48	0.47	0.42
F1	0.20	0.18	0.17	0.16	0.15	0.13	0.12	0.09
F2	−2.02	−0.78	−0.52	−0.38	−0.28	−0.22	−0.17	−0.05
F3	0.31	0.29	0.28	0.26	0.24	0.22	0.20	0.13
F4	0.53	0.51	0.48	0.46	0.43	0.41	0.38	0.28
F5	−0.10	−0.09	−0.08	−0.08	−0.07	−0.06	−0.06	−0.03

DISCUSSION

The relative prevalence of the different forms of diabetes in a cohort of 51 lean patients whose diagnosis was made before age 40, excluding typical IDDM, was investigated. Obese patients were excluded to eliminate a potential confounding effect of body weight on glucose metabolism. This is an important point, inasmuch as diabetic patients presenting before the age of 40 tend to be more obese than those presenting at an older age (29). Based on clinical criteria, patients were subdivided into a MODY and a non-MODY group. Serological and genetic analyses were then used to differentiate between genetically distinct MODY classes, autoimmune diabetes, mitochondrial diabetes, and a remaining group in which no specific etiology could be recognized. The distribution of patients in these classes is shown in Table 4.

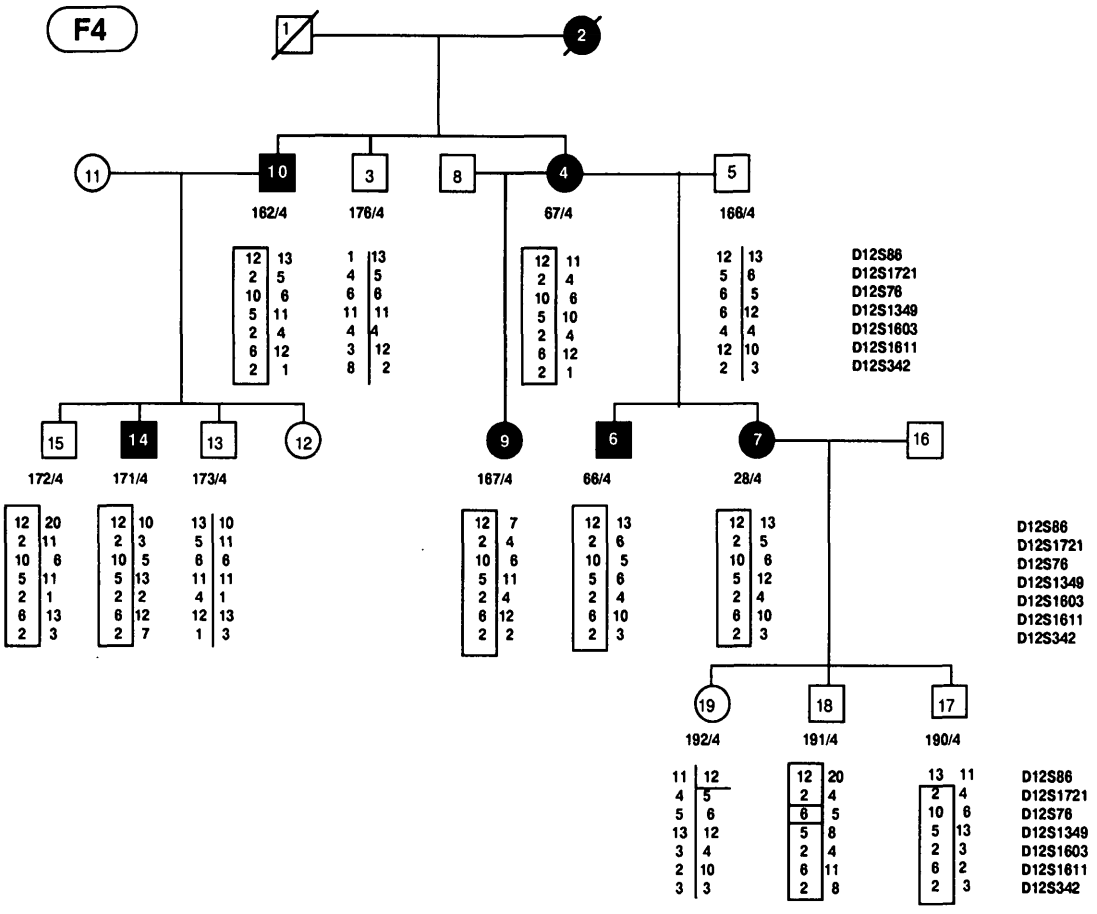

FIG. 3. Pedigree F4, demonstrating the chromosome 12q markers haplotype linked with the MODY phenotype.

Three subtypes of MODY, termed MODY1, MODY2, and MODY3, can currently be distinguished on the basis of the underlying genetic abnormalities (22,30,31). In the present study, we have identified four subjects with MODY2, defined as a disorder caused by mutations of glucokinase, the regulatory enzyme of carbohydrate metabolism (32). Although unrelated, these subjects all display the V203A mutation. To our knowledge, of >40 diabetes-related mutations previously reported, this particular mutation was identified in only one French family. The prevalence of the V203A mutation in the population tested here raises the possibility that the affected families originate from a single founder. One of the patients with the glucokinase mutation was originally classified in the non-MODY group because diabetes was diagnosed later than accepted by the definition. Even including this patient, the percentage of a glucokinase abnormality in the MODY group is <25%. Such a low percentage is at variance with that observed in France, where glucokinase mutations appear to occur in 60% of MODY cases (27), but similar to that found in the U.K. (33). These discrepancies in the prevalence of MODY2 are likely to be due to different recruiting strategies. The only conventional biological test that we found useful to distinguish between MODY2 and all other patients was a higher glucagon-stimulated C-peptide level in the former group. The finding of a well-preserved glucagon-stimulated insulin secretion in MODY2 is not entirely surprising, inasmuch as glucagon stimulates insulin secretion through a cAMP-dependent pathway (34), a process that does not directly implicate glucokinase.

Of the 17 remaining MODY patients, 5 subjects and their families were analyzed for linkage with the susceptibility gene on chromosome 12q (MODY3). One patient was identified with this form of diabetes. MODY3 may thus be responsible for one in five of MODY patients, although more precise figures will have to be derived from a larger number of representative families. Thus, ~60% of the present MODY patients remain genetically uncharacterized. They may be classified as MODYx, a provisional subclass probably comprising a variety of genetic abnormalities. Linkage of another form of MODY, termed MODY1, to an as-yet-unidentified gene located in the vicinity of the ADA gene on chromosome 20q has been reported in a large American pedigree of German ascent (30). Linkage to chromosome 20q has not been investigated in the present study, but the incidence worldwide of this form of the disease appears very low, and therefore it is unlikely to be the cause of diabetes in many of our patients. However, we must add that our study was not designed to specifically analyze MODY patients; our clinical criteria for definition of MODY were thus not robust, particularly the number of generations of family members affected. This point may explain the fact that less than half of the MODY patients could be characterized by the available genetic tests. The comparison of clinical data in the various MODY subclasses suggest that patients with MODY2 present a less severe form of diabetes than do MODY3 and MODYx patients.

Approximately 10% of the patients in this study had ICA titer exceeding 20 JDF U and thus appeared to be affected by an autoimmune form of diabetes. Five of these six patients

TABLE 3
Clinical and biological features in genetically distinct forms of MODY

Characteristics	MODY2 families	MODY3 family	MODYx families
Probands (*n*)	4	1	4
Probands + relatives (*n*)	9	5	17
Sex (M/F)	5 (56)/4 (44)	3 (60)/2 (40)	7 (41)/10 (59)
Age (years)	45.4 ± 16.5	35.8 ± 12.1	48.8 ± 13.2
BMI (kg/m^2)	22.4 ± 2.7	22.9 ± 2.3	24.3 ± 3.1
Age at diagnosis (years)	28.6 ± 14.2	25.4 ± 4.8	30.3 ± 13.3
Duration of diabetes (years)	17 ± 9.1	10.4 ± 11.8	18.4 ± 9.1
Presentation			
Incidental	6 (67)	3 (60)	9 (53)
Symptoms	0	0	3 (18)
Pregnancy	2 (22)	2 (40)	1 (6)
Other	1 (11)	0	0
Unknown	0	0	4 (23)
Fasting glycemia (mmol/l)	7.34 ± 1.48*	10.3 ± 2.7	9.0 ± 3.0
HbA_{1c} (%)	6.34 ± 0.80*	7.8 ± 0.1	7.8 ± 2.3
Basal C-peptide (nmol/l)	0.643 ± 0.263	0.428 ± 0.142	0.459 ± 0.125
Stimulated C-peptide (nmol/l)	1.544 ± 0.552*†	0.818 ± 0.222	0.789 ± 0.271
Increment of C-peptide (%)	148 ± 30‡	100 ± 71	76 ± 46
Treatment			
Diet alone	6 (67)†	2 (40)	1 (6)
Oral agent	2 (22)	2 (40)	7 (41)
Insulin	1 (11)§	1 (20)	9 (53)
Complications	0§	0	7 (41)

Data are means ± SE or *n* (%). MODY families are defined as families in which diabetes is transmitted as an autosomal dominant trait and diagnosed before 25 years of age in at least the youngest generation. MODY2 families are defined as the 4 probands and their relatives with the V203A glucokinase mutation (F10, F13, F27, and F45). In MODY3 families, diabetes in members of the family is linked to the chromosome 12q (F4). MODYx families are those in which the diabetes of probands and their relatives of the 4 MODY families is not linked to the glucokinase gene nor to the chromosome 12q marker (F1, F2, F3, and F5). *$P < 0.05$, MODY2 vs. MODY3 patients; †$P < 0.01$, MODY2 vs. MODYx patients; ‡$P < 0.001$, MODY2 vs. MODYx patients; §$P < 0.05$, MODY2 vs. MODYx patients.

were included in the clinically-defined non-MODY group; one of them fulfilled the clinical criteria of MODY. More than 2 years after the diagnosis, all six patients had levels of C-peptide in the low normal range, which explains the absence of absolute requirement of exogenous insulin. The diagnosis of diabetes in these patients was generally made at an older age compared with other patients (75th percentile of the whole population); no other parameter was clinically useful to distinguish these patients from those of the other subgroups. Surprisingly, none of the patients had anti-GAD antibodies.

Twenty-six patients included in the non-MODY group remained uncharacterized, and half of them were treated with insulin. Some of these patients might also have autoimmune diabetes with a lower titer of ICA. Of 14 patients with detectable ICA (>4 JDF U), 13 were indeed included in the non-MODY group. This low titer of ICA might reveal a latent autoimmune process in some of these patients. In addition, the prevalence of IDDM-associated HLA DQB1 alleles (either the 0302 allele or the presence of a nonaspartic residue in position 57) was higher in the non-MODY than in the MODY group. Furthermore, two of the three patients with GAD antibodies, who may also have autoimmune diabetes, were included in the non-MODY group. These results suggest that the prevalence of autoimmune diabetes is underestimated in our study and that the majority of patients with this form of diabetes are included in the non-MODY group, accounting for up to 50% of patients in this group.

A point mutation of nucleotide 3243 of the in mitochondrial DNA (tRNA $^{Leu(UUR)}$) was detected in a single patient. This mutation is responsible for rare forms of diabetes, known as maternally-inherited diabetes and deafness (MIDD), which do not strictly conform to the traditional definitions of IDDM

TABLE 4
Clinical and pathophysiological classification of diabetes in the young

		Clinical definition	
	n	MODY	non-MODY
n		19	32
MODY2	4	3 (15%)	1 (3%)
MODY3	1	1 (5%)	0
MIDD	1	1 (5%)	0
ICA$^+$	6	1 (5%)	5 (16%)
Unknown	39	13 (70%)	26 (81%)

Clinical MODY definition was diabetes diagnosed before 25 years of age, transmitted as an autosomal trait, and treated for at least 2 years without insulin. MODY2, patients with a mutation of the glucokinase gene; MODY3, linkage to the chromosome 12q; MIDD, maternally inherited diabetes and deafness due to the mitochondrial DNA mutation of nucleotide 3243 (tRNA $^{Leu(UUR)}$); ICA$^+$, patients with autoimmune diabetes (ICA > 20 JDF U); unknown, patients with diabetes not classified into subclasses described

and NIDDM (35,36). The present patient was initially included in the MODY group, although his family history was indeed compatible with maternal transmission. No hearing loss or muscular weakness was recorded in the physical examination.

Characterization of diabetes in the young adult using immunological and genetic parameters allowed us to classify 25% of the patients into specific diagnostic classes. Two important groups have been distinguished: patients with a glucokinase gene mutation and patients with autoimmune diabetes, potentially a slowly progressive IDDM. This differentiation, which was not possible using simple clinical parameters, has clinical, prognostic, and therapeutic implications. This distinction, however, presents clear limitations in our study due to lack of information on the family history of diabetes for multiple generations; this can only be done for members of informative families. Another limitation of the clinical classification is illustrated by the fact that a single genetic anomaly (MODY2) can cause both MODY and non-MODY as clinical phenotypes. Patients with MODY2 have "familial hyperglycemia" rather than diabetes, and metabolic control is achieved by diet alone in most of the cases (27). Autoimmune diabetes or slowly progressive IDDM, on the other hand, is a severe form of diabetes that will progress to insulin dependency. Identification of new immunological or genetic markers specific for the different forms of diabetes in the young adult should facilitate patient care. Early identification of diabetes based on these parameters will be important in the near future to initiate specific preventive strategies (37).

In conclusion, our data confirm that young and lean non-insulin-dependent diabetic patients constitute a very heterogeneous group, although they present similar clinical characteristics. Genetic and immunological testing clinically available at present may contribute to better classifying these patients according to pathophysiological criteria. MODY patients represent 40% of this population, but less than half of them can be characterized with the available genetic markers, which indicates that additional unknown genes are implicated in this disease. Autoimmune diabetes affects at least 12% of the patients, and its prevalence is probably underestimated. For a large majority of the patients, however, the etiology of diabetes remains to be determined.

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