Diabetes Complications in NIDDM Kindreds Linked to the MODY3 Locus on Chromosome 12q

GILBERTO VELHO, MD, PHD
MARTINE VAXILLAIRE, PHARMD, PHD
VALÉRIE BOCCIO
GUILLAUME CHARPENTIER, MD
PHILIPPE FROGUEL, MD, PHD

OBJECTIVE — To assess the prevalence of diabetes complications and the severity of diabetes in kindreds with NIDDM linked to the MODY3 locus (chromosome 12q) and to compare these parameters with data obtained in glucokinase (GCK)-deficient and other-MODY (unlinked to any of the three known loci) families, as well as with data from families with a late age of onset of NIDDM.

RESEARCH DESIGN AND METHODS — Clinical and biological data were obtained from 667 affected members of 7 MODY3, 25 GCK-deficient, 6 other-MODY, and 81 NIDDM families. Severity of diabetes (glucose tolerance status and insulin secretion) was assessed by an oral glucose tolerance test. Neurological examination and eye fundus examination were performed in 349 and 251 subjects, respectively, and proteinuria was tested with strips in 282 family members.

RESULTS — A higher prevalence of proliferative retinopathy was observed in MODY3 (21%) and NIDDM subjects (23%) than in GCK-deficient (3%) and other-MODY subjects (8%; $P = 0.004$). Proteinuria was detected in 19, 7, 5, and 0% ($P = 0.07$) of subjects, respectively. Prevalence of neuropathy was higher in NIDDM (17%; $P = 0.005$) than in MODY3 (4%), GCK-deficient (5%) and other-MODY (0%) subjects. MODY3 and NIDDM subjects had significantly higher fasting glucose levels than subjects in the other groups. Glucose levels after 2 h were significantly higher, and the ratios of insulin to glucose levels were significantly lower in MODY3 subjects than in the other three groups.

CONCLUSIONS — The MODY3 subtype of NIDDM is characterized by a severe insulin secretory defect and by major hyperglycemia that progresses rapidly to overt diabetes. Microvascular complications of diabetes were frequently observed in the MODY3 subjects and the subjects with a late age of onset of NIDDM in this cohort. Both the duration and the severity of diabetes were independently associated with these complications.

Maturity-onset diabetes of the young (MODY) is a familial subtype of NIDDM characterized by an early age of onset, usually before 25 years, and by autosomal dominant inheritance (1). The variable phenotype of subjects with MODY suggests that the disorder is genetically heterogeneous, an observation that has been confirmed by genetic studies. In one American family containing 66 affected diabetic individuals (RW pedigree), an as yet unidentified gene (MODY1) on the long arm of chromosome 20 was found to be linked to diabetes (2). On the other hand, we have observed that mutations in the glucokinase (GCK) gene on the short arm of chromosome 7 are responsible for diabetes in about half of the MODY families tested in France (3). We have recently localized to the long arm of chromosome 12 a third susceptibility locus to MODY in seven French kindreds (4,5). This localization has been confirmed in families from the U.K. (6), Germany, Denmark, and the U.S. (7). The MODY3 kindreds represent ~25% of our panel of MODY families; the remaining 25% of families are unlinked to the three known loci, implying that there is at least a fourth susceptibility locus to MODY. We have observed that diabetes in families linked to the MODY3 locus seems to be more severe than diabetes caused by GCK gene mutations (4). The aim of this work is to assess prevalence of diabetes complications and the severity of diabetes in MODY3 kindreds and compare these parameters with data obtained in GCK-deficient and other-MODY (unlinked to any of the three known loci) families, as well as with data from families with a late age of onset of NIDDM.

RESEARCH DESIGN AND METHODS

MODY and NIDDM families

These families belong to a panel collected from all over France (8). Clinical data were obtained by a standardized clinical examination performed at the Endocrinology Department of the Hôpital Saint-Louis or by the subject's personal physician. Neurological history was taken and a physical examination was performed focusing on the symptoms and signs of distal symmetric sensorimotor polyneuropathy and autonomic neuropathy. An eye fundus examination was performed by the subject's personal ophthalmologist. Proteinuria was tested with strips (lower limit of detection 2.5–3.0 mg/dl). The severity of diabetes (glucose tolerance and insulin secretion) was assessed by 75-g oral glucose tolerance test (OGTT). Subjects were considered affected if they had diabetes (fasting plasma glucose ≥7.8 mmol/l or 2-h post–oral glucose load ≥11.1 mmol/l), impaired glucose toler-

From the Institute Nacional de la Sante et de la Recherche Medicale U-358 (G.V.), Hôpital Saint-Louis, Paris; Service d'Endocrinologie (G.C.), Hôpital Gilles de Corbeil, Corbeil-Essonnes; and Centre National de la Recherche Scientifique EP10 (M.V., V.B., P.F.), Institut Pasteur/CHU, Lille, France.

Address correspondence and reprint requests to Gilberto Velho, MD, PhD, INSERM U358, Hôpital Saint-Louis, 16 rue de la Grange aux Belles, 75010 Paris, France.

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ANOVA, analysis of variance; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; MODY, maturity onset diabetes of the young.

Table 1—*Demographic, clinical, and biological profile of family members*

	MODY3	GCK	Other-MODY	NIDDM	*P*
Kindreds (*n*)	7	25	6	81	—
Glucose tolerance status					0.0001
MFH-IGT	2 (4)	108 (57)	16 (40)	86 (22)	
Diabetes	43 (96)	81 (43)	24 (60)	307 (78)	
Sex (M/F)	30/33	98/97	23/17	192/201	0.25
Age (years)	37 ± 18* (9–81)	37 ± 21* (2–96)	36 ± 22* (4–81)	59 ± 14 (26–92)	0.0001
BMI (kg/m^2)	22.5 ± 2.5*	21.9 ± 4.0*	23.5 ± 4.2*	27.1 ± 4.4	0.0001
Age at diagnosis (years)	23 ± 10* (9–48)	24 ± 17* (1–80)	26 ± 18* (3–79)	48 ± 13 (25–88)	0.0001
Duration of known hyperglycemia (years)	16 ± 12	12 ± 10†	10 ± 9†	11 ± 9†	0.01
Fasting glucose (mmol/l)	7.8 ± 2.8 (4.6–16.0)	7.0 ± 0.8† (4.3–16.5)	6.7 ± 1.5† (4.1–13.6)	8.8 ± 3.3 (4.6–22.9)	0.0001
Fasting insulin (mU/l)	11 ± 7* (3–35)	11 ± 7* (3–47)	10 ± 6* (3–32)	18 ± 8 (3–88)	0.0001
Fasting insulin/glucose (mU/mmol)	1.47 ± 1.18*	1.58 ± 0.94*	1.63 ± 0.99*	2.01 ± 1.31	0.004
2-h glucose (mmol/l)	15.9 ± 5.0 (7.7–23.0)	9.3 ± 2.9† (5.1–20.0)	9.6 ± 4.8† (4.4–20.7)	12.5 ± 4.6† (4.9–24.2)	0.0001
2-h insulin (mU/l)	29 ± 23* (5–79)	32 ± 19* (7–150)	36 ± 15* (6–97)	46 ± 27 (4–204)	0.0001
2-h insulin/glucose (mU/mmol)	1.76 ± 1.70	3.51 ± 1.92†	3.78 ± 2.81†	3.75 ± 3.10†	0.006
Treatment					0.0001
Diet	6 (13)	145 (77)	30 (75)	147 (37)	
OHA	33 (74)	41 (21)	10 (25)	211 (54)	
Insulin	6 (13)	3 (2)	0 (0)	35 (9)	

Data are means ± SD (range), *n*, or *n* (%). 2-h glucose and insulin are values after an OGTT. Statistics are contingency-table χ^2 test (qualitative traits) and ANOVA (quantitative traits). *$P < 0.05$, statistically significant difference compared to NIDDM; †$P < 0.05$, statistically significant difference compared to MODY3 (Tukey-Kramer test following ANOVA). MFH, mild fasting hyperglycemia (see METHODS for definition); OHA, oral hypoglycemic agents.

ance (IGT; 2-h post–oral glucose load ≥7.8 mmol/l), or a mild fasting hyperglycemia (plasma glucose ≥6.1 mmol/l) from two separate measurements. A fasting plasma glucose concentration ≥6.1 mmol/l represented a value 2–3 SD above the mean of the normal French population (9). Plasma glucose and creatinine were assessed at laboratories near the subject's home, while the assessment of plasma insulin was centralized at our laboratory. A total of 53 subjects out of 108 available family members of the seven MODY3 kindreds were found to carry diabetes-susceptibility haplotypes (4,5). These included 43 subjects with overt diabetes and 2 subjects with IGT, as well as 8 subjects with normal glucose tolerance. Table 1 shows demographic, clinical, and biological profiles of the affected individuals of MODY3, GCK-deficient, other-MODY, and late age of onset NIDDM families. For the sake of simplicity in results and the tables, NIDDM refers to subjects with a late age of onset of diabetes. Data of subjects who underwent examination for retinopathy, proteinuria, and neuropathy are shown in Table 2; all of these subjects had ≥5 years chronic hyperglycemia.

Data analysis

Data are expressed as means ± SD, unless otherwise stated. Quantitative traits were compared by Wilcoxon/Kruskal-Wallis tests and by analysis of variance (ANOVA). When ANOVA was significant, comparisons between pairs were made using Tukey-Kramer HSD test (10). Qualitative traits were analyzed by contingency-table χ^2 tests. Multivariate regression analyses and logistic regression analyses were performed to evaluate associations of clinical and biological parameters; for these analyses, data were normalized by logarithmic transformation. Statistics were generated with JMP software (SAS Inst., Cary, NC).

RESULTS

Complications of diabetes

We observed a significantly higher prevalence of proliferative retinopathy in MODY3 (21%) and NIDDM (23%) subjects than in GCK-deficient (3%) and other-MODY (8%) subjects ($P = 0.004$; Table 2). The average known duration of diabetes was higher, though not significantly different, in the MODY3 group compared with the other groups. To take this difference into account, we performed a logistic regression analysis with the retinopathy status as the categorical dependent variable and with the type of diabetes (MODY3, GCK-deficient, other-MODY, or NIDDM) and the known duration of diabetes as independent variables. Both parameters were independently associated with retinopathy ($P = 0.006$ and $P = 0.01$, respectively). The odds ratio for developing retinopathy in GCK-deficient and NIDDM subjects compared with MODY3 subjects was 0.27 (95% CI 0.08–0.92; $P = 0.03$) and 2.69 (95% CI 1.27–5.74; $P = 0.009$), respectively. Odds ratio estimation was not possible in the other-MODY group because of the small size of the sample. In a second analysis, fasting and 2-h glucose levels were included in the model as independent variables. Known duration of diabetes ($P = 0.04$), fasting glucose ($P = 0.01$), and 2-h glucose ($P = 0.03$), but not the type of diabetes ($P = 0.36$), remained independently associated with retinopathy. These results suggest that both the duration of diabetes and the severity of hyperglycemia in the MODY3 and NIDDM subjects are implicated in the higher prevalence of retinopathy in these groups of patients.

Table 2—*Retinopathy, proteinuria, and neuropathy in MODY and NIDDM subjects*

	MODY3	GCK	Other-MODY	NIDDM	*P*
Subjects tested for retinopathy					
n	24	65	12	150	
Sex (M/F)	8/16	27/38	6/6	71/79	0.56
Age (years)	44 ± 18*	46 ± 17*	49 ± 21*	62 ± 12	0.0001
Duration of known hyperglycemia (years)	23 ± 13	19 ± 11	18 ± 6	17 ± 9	0.06
Fasting glucose (mmol/l)	8.9 ± 2.6	7.2 ± 1.5*	6.4 ± 1.2*	9.7 ± 3.5	0.0001
Prevalence of proliferative retinopathy	5 (21)	2 (3)	1 (8)	34 (23)	0.004
Subjects tested for proteinuria					
n	26	78	12	166	
Sex (M/F)	11/15	38/40	5/7	86/80	0.45
Age (years)	42 ± 17*	45 ± 19*	53 ± 13	63 ± 12	0.0001
Duration of known hyperglycemia (years)	22 ± 13	18 ± 10	19 ± 5	18 ± 9	0.20
Fasting glucose (mmol/l)	8.7 ± 2.8	7.1 ± 0.9*	6.6 ± 1.4*	9.9 ± 3.7	0.0001
Systolic blood pressure (mmHg)	129 ± 11*	127 ± 17*	133 ± 18	140 ± 15	0.0001
Diastolic blood pressure (mmHg)	78 ± 7	74 ± 9*	70 ± 12*	80 ± 10	0.0001
Creatinine (μmol/l)	84 ± 28	84 ± 21	75 ± 17	86 ± 25	0.55
Prevalence of proteinuria	5 (19)	4 (5)	0 (0)	11 (7)	0.07
Subjects tested for neuropathy					
n	27	96	14	212	
Sex (M/F)	10/17	43/53	7/7	103/109	0.68
Age (years)	41 ± 16*	44 ± 19*	46 ± 21*	63 ± 12	0.0001
Duration of known hyperglycemia (years)	23 ± 13	17 ± 8†	17 ± 6	17 ± 8†	0.02
Fasting glucose (mmol/l)	8.6 ± 2.6	7.2 ± 1.3*	6.7 ± 1.3*	9.8 ± 3.5	0.0001
Prevalence of neuropathy	1 (4)	5 (5)	0 (0)	36 (17)	0.005

Data are means ± SD, *n*, or *n* (%). Statistics are contingency-table χ^2 test (qualitative traits) and ANOVA (quantitative traits). $P < 0.05$, statistically significant differences compared to NIDDM; †$P < 0.05$, statistically significant difference compared to MODY3 (Tukey-Kramer test following ANOVA).

MODY3 subjects with proliferative retinopathy were older (59 ± 17 vs. 39 ± 13 years; $P = 0.009$) and had a longer duration of diabetes (32 ± 16 vs. 20 ± 10 years; $P = 0.009$) and higher systolic blood pressure (141 ± 9 vs. 127 ± 11 mmHg; $P = 0.02$) and creatinine levels (111 ± 32 vs. 79 ± 15 μmol/l; $P = 0.03$) than MODY3 subjects without this complication. Two of them also presented with proteinuria. Plasma glucose and insulin levels, as well as other clinical and biological parameters, were not significantly different in the two groups of MODY3 subjects.

A trend toward a higher prevalence of nephropathy in MODY3 subjects than in subjects from the other three groups was observed ($P = 0.07$; Table 2): proteinuria was detected in 19, 7, 5, and 0% of the MODY3, NIDDM, GCK-deficient, and other-MODY subjects, respectively. This trend was confirmed in a logistic regression analysis with proteinuria status as the categorical dependent variable and with the type and known duration of diabetes as independent variables (data not shown; $P = 0.07$). Age, duration of diabetes, blood pressure, and plasma glucose, insulin, and creatinine levels were not significantly different in MODY3 subjects with or without proteinuria. Prevalence of neuropathy was significantly higher in the NIDDM group (17%; $P = 0.005$) than in the MODY3 (4%), GCK-deficient (5%), and other-MODY (0%) groups (Table 2). Both the type ($P = 0.02$) and the known duration of diabetes ($P = 0.0002$) were independently associated with neuropathy in a logistic regression analysis (data not shown).

Severity of diabetes

We evaluated three parameters: *1*) OGTT plasma glucose levels and glucose tolerance status, 2) insulin secretion during OGTT, and 3) the effect of the duration of diabetes on the insulin secretion defect and glucose tolerance status. MODY3-affected subjects had significantly higher fasting plasma glucose levels than GCK-deficient and other-MODY subjects (Table 1), and similar levels to NIDDM subjects. Plasma glucose levels after 2 h were significantly higher in MODY3 subjects than in the other three groups. These data agree with the observation of a significantly higher frequency of diabetes status, compared with the mild fasting hyperglycemia/IGT status, in MODY3 families than in the other groups of families. In this regard, MODY3 subjects were treated more often by oral hypoglycemic agents or insulin than subjects from the three other groups.

The 2-h insulin-to-glucose ratio was significantly lower in MODY3 subjects compared with the three other groups (Table 1), suggesting the presence of a more severe glucose-stimulated insulin secretory defect. Fasting plasma insulin, as well as the ratio of insulin to glucose levels, were not significantly different in the three groups of MODY, but were significantly higher in NIDDM subjects, probably reflecting some degree of insulin resistance in the latter group.

Multivariate regression analyses were performed, with the 2-h post-OGTT insulin-to-glucose ratio as the dependent variable and with age, age at diagnosis, known duration of diabetes, and BMI as independent variables. Data were normalized by log transformation for these

analyses. Duration of diabetes was significantly and independently associated with the severity of the insulin secretory defect in MODY3 and NIDDM subjects. This explained 37% ($P = 0.003$) and 30% ($P = 0.0001$) of the variance of 2-h post-OGTT insulin-to-glucose ratio in MODY3 and NIDDM groups, respectively, while no association was observed in the other two groups. Comparison within each group of the OGTT profiles of subjects with a short (≤10 years) or long (10 years) duration of diabetes confirmed these results (data not shown). MODY3 subjects with a long duration of diabetes presented a more severe insulin secretory defect (lower fasting and 2-h insulin levels and lower insulin-to-glucose ratios) and a more severely impaired glucose tolerance (higher fasting and 2-h glucose levels) than subjects with a short duration of diabetes (all comparisons, $P < 0.05$). Similar results were obtained for the NIDDM group. On the other hand, the insulin secretory defect and the glucose tolerance of GCK-deficient subjects and other-MODY subjects were found to be relatively stable. These results were confirmed by a cross-sectional analysis of the prevalence of overt diabetes as a function of the duration of known hyperglycemia. In the NIDDM group, the prevalences of overt diabetes in the intervals of 0–2, 2–4, 4–6, 6–8, and 8–10 years after diagnosis were 33, 52, 95, 100, and 96%, respectively. In the MODY3 group, the prevalence of diabetes was 71% for the 0- to 2-year interval following diagnosis and 100% for all other intervals. In the GCK-deficient group, the prevalences were 18, 24, 21, 51, and 38% for the same intervals; in the other-MODY group, they were 36, 17, 75, and 25% for the first four intervals. The prevalence of overt diabetes in the 5-year intervals from 10 to 30 years following diagnosis remained at 91–100, 100, 55–58, and 75–100%, respectively, in the NIDDM, MODY3, GCK-deficient, and other-MODY groups.

CONCLUSIONS — The genetic characterization of NIDDM families made it possible to describe the clinical and biological profiles of genetically homogeneous subtypes of diabetes. We have observed that MODY, which has often been considered a milder form of chronic hyperglycemia than late age of onset NIDDM, might not always be so. The clinical phenotype of MODY3 resembles late age of onset NIDDM in its natural history, with subjects rapidly progressing from IGT to overt diabetes and with the deterioration of insulin secretion. Proliferative retinopathy was observed as frequently in MODY3 as in late age of onset NIDDM. When adjusted for the duration of diabetes, the odds ratio to develop retinopathy was intermediate in MODY3 subjects, compared with GCK-deficient (3.0 times as low) and late age of onset NIDDM (2.6 times as high) subjects. A trend toward a higher prevalence of proteinuria in MODY3 subjects was also observed. However, unlike NIDDM with a late age of onset, the MODY3 subtype is associated with a low prevalence of obesity, dyslipidemia, and arterial hypertension (data not shown). In this regard, affected individuals from the RW family in which MODY was found to be linked to the MODY1 locus on chromosome 20 presented a severe form of diabetes that requires insulin therapy in ~30% of cases and is associated with microvascular complications (11).

It should be pointed out that the design of our study has intrinsic limitations that might have affected the results of the prevalence of diabetes complications. The 119 families that participated in this study (667 diabetic family members) belonged to a panel of multiplex diabetic kindreds that were collected from all over France to study the genetic determinants of NIDDM and IDDM (8). Because of the large geographical dispersion of these families, >80% of the subjects were not examined at the Endocrinology Department of Hôpital Saint-Louis in Paris. Instead, each of these subjects had a standardized clinical examination performed by his or her own personal physician and an eye fundus examination performed by his or her own ophthalmologist. In this way, the diagnoses of diabetes complications were made by numerous different observers at numerous clinics. To lessen the impact of this possible source of bias, only proliferative retinopathy was considered in this study. Because 82% of the subjects who were reported to present proliferative retinopathy were known cases who had already received laser treatment, we estimated that the rate of false-positive diagnosis in this group is probably low. Regarding the assessment of proteinuria, the dipstick method is influenced by factors such as urinary concentration and thus subject to error. On the other hand, because this method is insensitive to early changes such as microalbuminuria, the prevalence of nephropathy may have been underestimated. The prevalence of peripheral neuropathy might also have been underestimated because the diagnosis of this complication was based on the clinical examination alone; no nerve conduction study was systematically performed. For all these reasons, our results should only be considered as a relative estimate in these four groups of families of the prevalence of diabetes complications in subjects with ≥5 years of chronic hyperglycemia.

The diabetes susceptibility gene on the MODY3 locus probably controls an important step in insulin secretion (12). Identification of the gene product and its defect will not only further the understanding of the mechanisms of insulin secretion and help to elucidate the pathophysiology of this subtype of NIDDM, but will also provide the diagnostic tools to recognize subjects carrying the diabetes-susceptibility allele. At the moment, this is possible only by linkage analyses in large pedigrees. Early therapeutic intervention in these subjects might be essential to prevent diabetic complications.

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References

1. Fajans SS: Scope and heterogeneous nature of MODY. *Diabetes Care* 13:49–64, 1990
2. Bell GI, Xiang KS, Newman MV, Wu SH, Wright LG, Fajans SS, Cox NJ: Gene for non insulin dependent diabetes mellitus (maturity onset diabetes of the young subtype) is linked to DNA polymorphism on chromosome 20q. *Proc Natl Acad Sci USA* 88:1484–1488, 1981
3. Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffel M, Takeda J, Passa P, Permutt MA, Beckman JS, Bell GI, Cohen D: Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *N Engl J Med* 328:697–702, 1993
4. Vaxillaire M, Boccio V, Philippi A, Vigour-

oux C, Terwilliger J, Passa P, Beckman JS, Velho G, Lathrop GM, Froguel P: A gene for maturity onset diabetes of the young (MODY) maps to chromosome 12q. *Nature Genet* 9:418–423, 1995
5. Froguel P, Velho G: Genetic and metabolic heterogeneity of maturity onset diabetes of the young. *Diabetes Rev.* In press
6. Dronsfield MJ, Tack CJJ, Bain SC, Hattersley AT: Linkage of the gene for maturity-onset diabetes of the young with markers on the long arm of chromosome 12q (Abstract: European Association for the Study of Diabetes, 31st Annual Meeting, Stockholm, Sweden, September 1995). *Diabetologia* 38 (Suppl. 1):A60, 1995
7. Menzel S, Yamagata K, Trabb JB, Nerup J, Permutt MA, Fajans SS, Menzel R, Iwasaki N, Omori Y, Cox NJ, Bell GI: Localization of MODY3 to a 5-cM region of human chromosome 12. *Diabetes* 44:1408–1413, 1995
8. Froguel P, Velho G, Cohen D, Passa P: Strategies for the collection sibling-pair data for genetic studies in type 2 (non insulin-dependent) diabetes mellitus. *Diabetologia* 34:685, 1991
9. Tchobroutsky G: Blood glucose levels in diabetic and non diabetic subjects. *Diabetologia* 34:67–73, 1991
10. Kramer CY: Extension of multiple range tests to group means with unequal number of replications. *Biometrics* 12:309–310, 1956
11. Fajans SS, Bell GI, Bowden DW, Halter JB, Polonsky KS: Maturity-onset diabetes of the young. *Life Science* 55:413–422, 1994
12. Velho G, Clément K, Pueyo ME, Vaxillaire M, Passa P, Robert JJ, Froguel P: Clinical and metabolic profiles of MODY families linked to markers on chromosome 12q (Abstract: European Association for the Study of Diabetes, 31st Annual Meeting, Stockholm, Sweden, September 1995). *Diabetologia* 38 (Suppl. 1):A34, 1995