Phenotypic Characteristics of Early-Onset Autosomal-Dominant Type 2 Diabetes Unlinked to Known Maturity-Onset Diabetes of the Young (MODY) Genes

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OBJECTIVE — To investigate whether there are forms of early-onset autosomal-dominant type 2 diabetes that are distinct from typical maturity-onset diabetes of the young (MODY) and to characterize their phenotypic characteristics.

RESEARCH DESIGN AND METHODS — The study included 220 affected subjects from 29 families in which early-onset type 2 diabetes occurred in multiple generations and was not linked to known MODY genes (MODY gene–negative families). All individuals underwent an oral glucose tolerance test and other clinical measurements aimed at investigating the underlying metabolic defect and the presence of diabetic complications. For comparison, 79 affected carriers of MODY3 (hepatocyte nuclear factor [HNF]-1α) mutations were similarly examined.

RESULTS — Subjects from MODY gene–negative pedigrees were diagnosed with diabetes at an older age (36 ± 17 vs. 21 ± 10 years, $P = 0.0001$) and were more frequently obese (52 vs. 18%, $P = 0.0001$) than MODY3 individuals. MODY gene–negative patients who were insulin treated required more exogenous insulin than did MODY3 subjects (0.7 ± 0.4 vs. 0.45 ± 0.2 U · kg^{-1} · day^{-1}, $P = 0.04$), despite similar C-peptide levels. Among subjects not treated with insulin, MODY gene–negative subjects had significantly higher serum insulin levels, both fasting (16.5 ± 15 vs. 6.5 ± 5 μU/ml, $P = 0.027$) and 2 h after a glucose load (53 ± 44 vs. 11 ± 10, $P = 0.002$). They also had higher serum triglycerides ($P = 0.02$), higher cholesterol levels ($P = 0.02$), more hypertension ($P = 0.0001$), and more nephropathy ($P = 0.001$). Differences persisted when families were matched for age at diagnosis.

CONCLUSIONS — Our findings indicate the existence of forms of early-onset autosomal-dominant type 2 diabetes that are distinct from MODY and are frequently characterized by insulin resistance, similar to later-onset type 2 diabetes. Because of the Mendelian pattern of inheritance, the goal of identifying the genes involved in these forms of diabetes appears to be particularly feasible.

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Abbreviations: ACR, albumin-to-creatinine ratio; GDM, gestational diabetes mellitus; HNF, hepatocyte nuclear factor; IA, insulinoma-associated protein; IGT, impaired glucose tolerance; IPF, insulin-promoter factor; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; OR, odds ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Genetic factors play a crucial role in the etiology of type 2 diabetes (1,2). However, efforts to identify type 2 diabetes genes have not been successful because of the disease's complexity and heterogeneity (3). One approach taken to identify type 2 diabetes genes has been the study of maturity-onset diabetes of the young (MODY), a relatively rare type of familial diabetes characterized by a young age at onset, often before age 25, and an autosomal mode of inheritance (4). This approach—studying a rare, single gene form of a disorder to infer about more common and complex varieties—is similar to that used for breast cancer, which has led to the identification of the breast cancer genes BRCA1 and BRCA2 (5,6). The presence of large families with multiple affected members has facilitated studies of MODY. Five distinct MODY genes—glucokinase, hepatocyte nuclear factor (HNF)-1α, -4α, and -1β, and insulin-promoter factor (IPF)-1—have been identified so far in different pedigrees (7–13).

Although successful identification of the MODY genes has provided new insights into the pathophysiology of β-cells, it has not helped to identify genes involved in the etiology of insulin resistance and common type 2 diabetes. Several studies have failed to detect linkage or association between the glucokinase locus and type 2 diabetes (3). Similarly, genetic variations at the HNF-1α and HNF-4α loci do not appear to contribute to common, late-onset type 2 diabetes (14–16). The reason for such negative findings is that MODY, being characterized by a pure insulin secretion defect rather than by an impairment of insulin sensitivity, is basically a different disorder from type 2 diabetes (2,17–19). A better strategy to identify type 2 diabetes genes would be to study other forms of early-onset autosomal-dominant type 2 diabetes that—while having a strong genetic component and a simple pattern of inheritance—may be pathophysiologically more similar to "garden variety" type 2 diabetes than is MODY.

The existence of such forms of early-onset type 2 diabetes has been postulated in the past, but distinguishing them from MODY has been difficult because of the considerable overlap of clinical features (20–22). With the recent identification of MODY genes, it has now become possible to investigate whether families carrying these forms of type 2 diabetes indeed exist and to define their phenotype. To this end, we describe here the clinical features of diabetes in 29 families with early-onset type 2 diabetes transmitted in an autosomal-dominant fashion and not due to known MODY genes as compared with those of 10 kindred with MODY caused by mutations in the HNF-1α gene. Our findings indicate that forms of early-onset autosomal-dominant type 2 diabetes that are not linked to known MODY genes are relatively frequent and are clinically distinct from MODY, being frequently characterized by insulin resistance and a higher risk of long-term complications.

RESEARCH DESIGN AND METHODS

Ascertainment and examination of families

Families with an autosomal-dominant pattern of occurrence of early-onset type 2 diabetes were ascertained using the following screening criteria: *1*) a proband and at least one first-degree relative with type 2 diabetes diagnosed before age 35, *2*) three or more generations affected by diabetes, and *3*) diabetes entering the family on only one side. An age at diagnosis of 35 rather than 25 years was chosen as a cutoff to allow for the fact that in ~25% of the cases, MODY is diagnosed after the traditional age limit of 25 years. To develop a roster of families meeting these criteria, several approaches were used: we screened 2,700 Joslin patients who had diabetes diagnosed before age 35, placed advertisements seeking such families in journals that target 1.5 million patients with diabetes in the U.S. and Canada, and sent a similar advertisement to 9,000 diabetes educators in the U.S. Through these efforts, we identified ~1,000 probands who reported early-onset autosomal-dominant type 2 diabetes in their families. The families were screened further by telephone and correspondence to find out about the occurrence of diabetes in the extended pedigrees and find out whether the family was willing to participate in a genetic study.

The study protocol and informed consent procedures were approved by the Human Subjects Committee of the Joslin Diabetes Center. After giving written consent to participate, individual family members living in New England were examined by specially trained family recruiters. Members of eligible families living in other parts of the U.S. or Canada were examined by nurses and phlebotomists in local medical facilities. Examinations were performed in the fasting state and included a medical questionnaire and measurement of height and weight. Among other questions, subjects were asked whether and in which year they had been diagnosed with diabetes by a physician, the type of diabetes, and the starting date and current dose of glucose-lowering treatment. In the case of ambiguous or incomplete information, clinical data were obtained from family doctors.

Fasting blood was drawn for blood glucose determinations and DNA extraction. Serum was obtained for C-peptide determination for insulin-treated diabetic individuals. Nondiabetic individuals and those with diabetes treated with oral agents or diet had an additional blood sample drawn 2 h after an oral challenge with 75 g of glucose for blood glucose determination and other biochemical measurements.

Diabetes was diagnosed *1*) if an individual was treated with insulin or oral agents; *2*) if results of the oral glucose tolerance test (OGTT) met World Health Organization criteria (blood glucose >140 mg/dl fasting or >200 mg/dl at 2 h); or *3*) if the level of HbA_{1c} was >7.0% in individuals who declined the OGTT or were not fasting when examined. Impaired glucose tolerance (IGT) was defined as a blood glucose <140 mg/dl fasting and between 140 and 200 mg/dl at 2 h during the OGTT. Previous gestational diabetes mellitus (GDM) was diagnosed on the basis of medical history according to World Health Organization criteria (23).

Clinical evaluation

Information on the medical history of individual family members was obtained by questionnaire and interview at the time of the examination. Among other questions, participants were asked for their lifetime maximum body weight, blood pressure values at the last doctor visit, history of physician-diagnosed hypertension and antihypertensive therapy, kidney disease, laser treatment of their eyes, diagnoses of heart attack or angina by a physician, and whether the heart condition had ever required surgical treatment including coronary angioplasty. Ambiguous or incomplete information was clarified by contacting family doctors.

Diagnoses of diabetic complications

The diagnosis of coronary artery disease was based on medical history. Hypertension was diagnosed if individuals were currently receiving antihypertensive drugs or if their systolic or diastolic blood pressure was ≥140 or 90 mmHg, respectively. Proliferative retinopathy was diagnosed on the basis of a history of laser treatment. Diabetic nephropathy was diagnosed as previously described (24). A random urine sample obtained at the time of examination was screened for overt albuminuria with reagent strips (Albustix; Bayer, Elkhart, IN) read by an optical scanner. If the sample was not strongly positive for overt albuminuria (2+), the urinary albumin-to-creatinine ratio (ACR) was measured. Normoalbuminuria was defined as an ACR <17 μg/mg for men and <25 μg/mg for women. Overt proteinuria was defined as an ACR ≥250 μg/mg for men or ≥355 μg/mg for women or a reagent strip reading ≥2+. Microalbuminuria was defined as an ACR in the range between normoalbuminuria and overt proteinuria.

Laboratory methods

For examinations in New England, the family recruiters used home glucose meters (One Touch; Lifescan, Milpitas, CA) to determine glucose values. For examinations performed elsewhere, blood glucose was determined by the methods of the local laboratories. For these samples, the method used was recorded, and the glucose measurements were re-expressed in terms of one reference method by means of appropriate conversion formulas. Serum insulin and C-peptide were measured by radioimmunoassay (Linco Research, St. Charles, MO). HbA_{1c} was measured by high-performance liquid chromatography in the Clinical Laboratory of the Joslin Diabetes Center. Fasting total serum cholesterol, triglycerides, and HDL cholesterol were measured by an enzymatic method using the Beckman Synchron CX System (Beckman Instruments, Brea, CA). Urinary albumin concentration was measured by immunonephelometry with N Albumin kits (Behring, Somerville, NJ). Urinary creatinine concentrations were measured by colorimetry (modified Jaffé's reaction) on an Astra-7 automated system (Beckman).

Table 1—*Characteristics of examined families*

Family number	Affected subjects (*n*)	Median age at diagnosis (range)
MODY3 families		
1	5	12 (10–34)
2	4	12 (10–13)
3	3	13 (10–18)
4	13	15 (8–48)
5	16	15.5 (9–37)
6	4 (4)	16.5 (15–40)
7	10 (1)	17.5 (11–41)
8	7 (2)	19.5 (19–47)
9	11 (2)	20 (14–53)
10	6	21.5 (17–34)
Total	79 (9)	18 (8–53)
MODY gene–negative families		
11	5	10 (3–55)
12	5	14 (3–17)
13	10 (3)	14.5 (11–57)
14	7(1)	19 (11–77)
15	6 (4)	20.5 (5–43)
16	10	21 (11–49)
17	4	29.5 (24–71)
18	7 (3)	30 (13–57)
19	7 (1)	30 (3–47)
20	4	30.5 (14–41)
21	7	31 (13–51)
22	8 (1)	32.5 (25–65)
23	7 (1)	33 (26–55)
24	7	33 (6–72)
25	7 (1)	33 (9–76)
26	8 (1)	35.5 (13–48)
27	6 (1)	36.5 (26–56)
28	9 (1)	37 (27–51)
29	4	38 (27–50)
30	5	39 (26–48)
31	9	39.5 (9–71)
32	6 (1)	39.5 (17–51)
33	11 (4)	40 (13–51)
34	9	41 (11–65)
35	6 (1)	42.5 (12–55)
36	12 (1)	42.5 (14–62)
37	12 (2)	42.5 (15–88)
38	13 (1)	44 (2–70)
39	9 (1)	44.5 (27–59)
Total	220 (29)	36 (2–88)

The numbers of subjects with IGT or previous GDM are indicated in parentheses. The four members of MODY3 family number 6 reported a diagnosis of diabetes but were found to have only IGT at examination.

Serum anti-GAD and anti–insulinoma-associated protein–2 (IA-2) antibody levels were measured by radioligand assays using recombinant GAD and IA-2 proteins (25,26). Results were expressed as counts per minute indexes. An index >0.1 (2 SD above the mean in normal control subjects) was considered positive for both antibodies.

Classification of families

A total of 43 families meeting the above criteria were ascertained in 1995 and 1996. All families were of Caucasian origin, with the exception of three Hispanic and two African-American pedigrees. All families were screened for mutations in the HNF-4α (MODY1) and HNF-1α (MODY3) genes segregating with diabetes and examined for linkage with the glucokinase locus (MODY2). Mutations in these three genes account for the vast majority of MODY cases due to known MODY genes, as MODY4 (IPF-1) and MODY5 (HNF-1β) have been thus far described only in single families (12,13). As previously described (27), HNF-1α (MODY3) mutations linked with diabetes were searched in these families by means of double gradient, denaturing gradient gel electrophoresis, followed by direct sequencing of products of polymerase chain reaction that were amplified from the HNF-1α exons and the promoter. Missense or frameshift mutations were identified in 10 of these families (27), hereafter designated as MODY3 families. The remaining 33 families were also screened for mutations in the HNF-4α gene (MODY1) using the same strategy used for HNF-1α (M.M., A.S.K., unpublished observations). After double gradient, denaturing gradient gel electrophoresis and sequencing of all 12 HNF-4α exons and the promoter, none of the pedigrees exhibited sequence differences segregating with diabetes. Finally, linkage with markers HGKCA1 and D7S2428 flanking the glucokinase locus (MODY2) was also excluded in these families by parametric linkage analysis with logarithm of odds scores of −9.1 and −28.7, respectively, and no significant evidence of linkage heterogeneity (A.D., unpublished observations). These 33 pedigrees are hereafter designated as MODY gene–negative families.

Four of the MODY gene–negative pedigrees were excluded from the present study because the examinations of family members have not been completed. The remaining 39 families (10 MODY3 and 29 MODY gene–negative pedigrees) included 707 members who were alive as of July 31, 1997. A total of 194 of these individuals, 47 of whom were reported as affected by diabetes by other family members, declined to participate in this study or could not be traced or examined. The remaining 513 family members were examined according to the above protocol: 214 had normal glucose tolerance, 261 were diagnosed with diabetes, 30 had IGT, and 8 had been previously diagnosed with GDM. All individuals with diabetes, IGT, or previous GDM were included in the present study. The MODY3 group included a total of 79 individuals (70 with diabetes and 9 with either IGT or previous GDM). The MODY gene–negative groups consisted of 220 subjects (191 with diabetes and 29 with IGT or previous GDM).

Statistical analysis

To take into account the lack of independence of observations on multiple family members, differences among study groups were tested by means of generalized estimating equations (GEE) using the SAS package (SAS Institute, Cary, NC). Sex, age at examination, and time elapsed since diabetes diagnosis were incorporated in the statistical models. As a descriptive measure of association, odds ratios (ORs) were calculated along with 95% CIs (28).

RESULTS

Age at diagnosis of diabetes

A total of 39 families with an autosomal-dominant pattern of occurrence of early-onset type 2 diabetes were included in this study. In 10 of these families, diabetes segregated with mutations in the HNF-1α gene (MODY3 families). Diabetes was not related to known MODY genes in the remaining 29 pedigrees (MODY gene–negative families). Table 1 summarizes some individual characteristics of the examined families ranked by their median age at diagnosis of diabetes. Overall, individuals from MODY gene–negative pedigrees were diagnosed with diabetes at an older age (median = 36 years) than were MODY3 family members (median = 18 years). There was, however, large variability in the age at diagnosis both within and among families, resulting in a considerable overlap between the two groups of families. In particular, 6 of the MODY gene–negative pedigrees (families 11 through 16 in Table 1) had a distribution of age at diagnosis quite similar to that of the 10 MODY3 families. To assess whether this particularly young age at diagnosis was associated with some distinctive clinical features, these six families were considered separately from the

Table 2—*Clinical characteristics of the affected members of families with early-onset autosomal-dominant type 2 diabetes*

	MODY3 families	MODY gene–negative families: Median age at diagnosis <25 years	MODY gene–negative families: Median age at diagnosis ≥25 years	*P* value
Families (*n*)	10	6	23	
Subjects (M/W)	79 (32/47)	43 (14/29)	177 (64/113)	0.66*
IGT/GDM (%)	11.4	18.6	11.9	0.45
Age at diabetes diagnosis (years)	21 ± 10	25 ± 18	39 ± 16	
Age at examination (years)	38 ± 17	42 ± 19	50 ± 18	0.0001†
Time from diabetes diagnosis (years)	18 ± 15	17 ± 16	12 ± 11	0.006†
Percent ideal body weight at examination	113 ± 21	121 ± 27	143 ± 36	0.0001‡
Lifetime maximum percent ideal body weight	122 ± 22	133 ± 32	158 ± 43	0.0001‡
Obesity (%)	17.8	29.7	56.7	0.0001
Type of treatment				0.52
Diet alone (%)	30.4	23.2	28.8	—
Oral agents (%)	22.8	25.6	31.1	—
Insulin (%)	46.8	51.2	40.1	—
β-Cell antibody positive (%)	7.8	15.4	11.0	0.56

Data for quantitative variables are means ± SD. For β-cell antibody positive, anti-GAD and anti-A2 antibodies were measured in 64, 26, and 154 individuals in the three study groups, respectively. *The *P* value refers to differences in sex distribution across the three groups; †*P* values after controlling for differences in sex distributions; ‡*P* values are after controlling for differences in sex, age at examination, and time elapsed since diabetes diagnosis.

other MODY gene–negative families in the subsequent analysis.

Clinical features of diabetes

Selected clinical characteristics of the study subjects are summarized in Table 2. The MODY gene–negative group with a median age at diagnosis older than 25 years included 40 non-Caucasian subjects whose clinical features were not significantly different from those of the Caucasian subjects in this group. Besides being diagnosed at an older age, individuals from MODY gene–negative families were currently heavier than subjects from MODY3 families (P = 0.0001 after controlling for differences in sex, age at examination, and time elapsed since diabetes diagnosis). This difference, however, was especially evident for the MODY gene–negative group of families with a median age at diagnosis older than 25 years. A similar pattern was observed for the maximum lifetime body weight (P = 0.0001). The prevalence of obesity (defined as a current percent of ideal body weight >130) was 52% (95% CI: 47.0–60.5%) in the MODY gene–negative families (56.7% [49.4–64.0] in the group with an older age at diagnosis and 29.7% [16.0–43.4] in the group with a younger age at diagnosis) as compared with 17.8% (9.4–26.2) among the MODY3 pedigrees (P = 0.0001, after adjusting for differences in sex, age at examination, and duration of diabetes). The prevalence of individuals with IGT or previous GDM rather than overt diabetes was slightly higher in the MODY gene–negative groups than among MODY3 families, but this difference was not significant (P = 0.45). A similar treatment pattern was observed in the three family groups (P = 0.52), with between 40 and 50% of the individuals requiring insulin therapy. A number of individuals positive for β-cell antibodies, either anti-GAD or anti-IA-2, were detected in both MODY gene–negative groups, but their prevalence (11.0% in the older and 15.4% in the younger at diagnosis subgroups) was not significantly different from that observed in the MODY3 families (7.8%, P = 0.56). Within each family group, antibody-positive subjects were characterized by a younger age at diagnosis, a lower body weight, and a higher prevalence of insulin treatment than were antibody-negative individuals (data not shown).

Indices of insulin secretion and insulin sensitivity are in Table 3, shown separately for insulin-treated and non-insulin-treated subjects. Individuals from both MODY gene–negative groups required more exogenous insulin to control their diabetes than did MODY3 subjects (P = 0.04), while exhibiting similar fasting C-peptide levels (P = 0.51) and a similar prevalence of undetectable C-peptide (P = 0.50). The suggestion that members of the MODY gene–negative families were, on average, insulin resistant in comparison with MODY3 individuals was confirmed among non-insulin-treated subjects. Among these individuals, members of MODY gene–negative families had significantly higher fasting insulin levels than did MODY3 individuals (16.5 ± 15 in the overall MODY gene–negative group, and 16.9 ± 16.0 and 14.6 ± 7.4 μU/ml in the older and younger subgroups, respectively, versus 6.5 ± 5.2 μU/ml in the MODY3 group, P = 0.027). This difference was not significant when body weight was taken into account (P = 0.23), indicating that the higher fasting insulin levels were probably due to the higher prevalence of obesity in the MODY gene–negative groups. Serum insulin levels rose to 53.2 ± 44 μU/ml in the MODY gene–negative families 2 h after the glucose load (54.3 ± 45 μU/ml in the families with age at diagnosis older than 25 years and 46.3 ± 47 μU/ml in families with younger age at diagnosis) as compared with only 11.4 ± 10 μU/ml in the MODY3 families (P = 0.002). Differences were independent of the time since diabetes diagnosis, type of treatment (diet alone or oral agents), or obesity. These differences were particularly striking when the individual insulin increments were examined in relation to 2-h glucose increments during OGTT. As illustrated in Fig. 1, the difference between 2-h and fasting insulin levels was modest—less than 20 μU/ml—in all MODY3 individuals, regardless of their 2-h glucose excursions. In contrast, a more heterogeneous pattern was observed among the MODY gene–negative individuals, similar to what is commonly observed in later-onset type 2 diabetes. Although individuals with a poor insulin response were also present in the MODY gene–negative group, many of these subjects had a difference between fasting and 2-h insulin levels that exceeded 20 μU/ml for any 2-h glucose increment, reaching values >100 μU/ml in some individuals. Taken together, these results suggested that insulin resistance was a frequent

Table 3—*Indices of insulin secretion and insulin sensitivity in the three groups of families with early-onset autosomal-dominant type 2 diabetes*

	MODY3 families	MODY gene–negative families: Median age at diagnosis <25 years	MODY gene–negative families: Median age at diagnosis ≥25 years	*P* value
Insulin-treated subjects				
C-peptide negative (%)	16.7	33.3	22.7	0.50
Fasting serum C-peptide (ng/ml)	0.84 ± 0.6	1.15 ± 0.7	1.32 ± 0.9	0.51
Insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$)	0.45 ± 0.2	0.62 ± 0.3	0.72 ± 0.4	0.04
Non–insulin-treated subjects				
Fasting blood glucose (mg/dl)	116 ± 37	145 ± 61	144 ± 56	0.13
2-h blood glucose (mg/dl)	259 ± 97	240 ± 113	245 ± 102	0.74
Fasting serum insulin (μU/ml)	6.5 ± 5.2	14.3 ± 7.4	16.9 ± 15.5	0.027
2-h serum insulin (μU/ml)	11.4 ± 9.9	46.3 ± 47	54.3 ± 44.0	0.002
HbA_{1c} (%)	6.9 ± 1.6	7.0 ± 1.5	7.5 ± 1.6	0.26

Data for quantitative variables are means ± SD. *P* values were calculated after controlling for differences in sex, age at examination, and time elapsed since diabetes diagnosis. Fasting serum C-peptide values were available for 30, 12, and 66 individuals in the three study groups, respectively. For fasting serum C-peptide, means refer to individuals who had measurable C-peptide levels. Data for non–insulin-treated subjects are from 29 MODY3 and 78 MODY gene–negative individuals (11 from the youngest age at diagnosis subgroup, 67 from the oldest) who were not insulin treated and had all five variables measured during OGTT.

feature of diabetes in the MODY gene–negative families.

Diabetic complications

Serum lipid levels and the prevalence of hypertension and micro-/macrovascular complications of diabetes in the study groups are reported in Table 4. Consistent with the presence of insulin resistance, individuals from MODY gene–negative pedigrees had higher serum triglycerides and lower levels of HDL cholesterol than did MODY3 individuals ($P = 0.020$ and $P = 0.17$, respectively). They also had higher levels of total serum cholesterol ($P = 0.019$). Similar to fasting insulin levels, differences in lipid levels decreased and lost significance when data were adjusted for body weight, suggesting that these features were closely related to obesity. MODY gene–negative families were also characterized by a much higher prevalence of hypertension ($P = 0.0001$). This was especially evident for the group with age at diagnosis older than 25 years, in which the prevalence of hypertension reached 63%. After adjusting for differences in sex, age, and duration of diabetes, members of this group of families were eight times more likely to be hypertensive than subjects from MODY3 pedigrees (OR = 8.3 [3.2–20.8]). The risk of hypertension in the MODY gene–negative group with younger age at diagnosis was lower, but still higher than that in MODY3 pedigrees (OR = 2.5 [0.7–9.2]). Similar to the findings for hypertension, MODY gene–negative families showed a higher prevalence of diabetic nephropathy than did MODY3 pedigrees ($P = 0.001$), despite a shorter or identical time from diagnosis of diabetes (Table 1). Independent from their age at diagnosis or sex, the risk of nephropathy for MODY gene–negative individuals was three times higher than that among MODY3 individuals (ORs = 3.0 [1.8–5.2] and 3.0 [1.3–7.3] for the older and younger age at diagnosis groups, respectively). Similar risk estimates were obtained if different degrees of nephropathy (microalbuminuria or overt proteinuria) were considered or if study subjects were stratified according to their

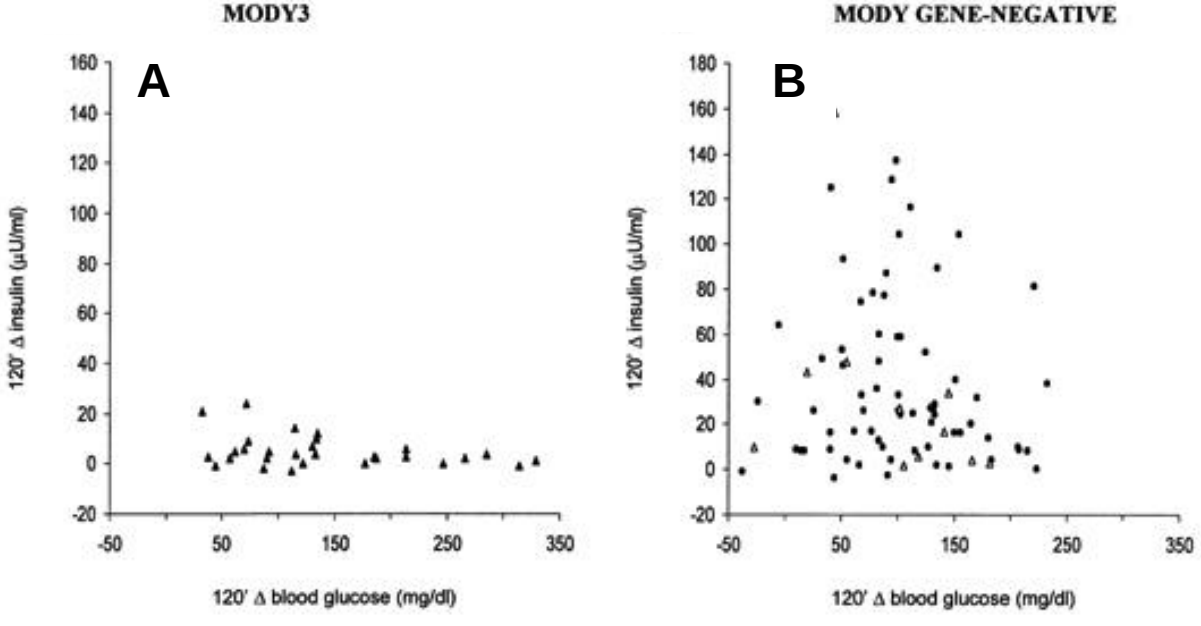

Figure 1—*Relationship between the increment in insulin secretion 2-h after OGTT (ΔIns [insulin; μU/ml]) and A: the 2-h glucose increments during the test among MODY3 individuals (▲) or B: MODY gene–negative subjects (●, the older age at diagnosis subgroup; △, the younger age at diagnosis subgroup).*

Table 4—*Serum lipids and prevalence of hypertension and diabetic complications in three groups of families with early-onset autosomal-dominant type 2 diabetes*

	MODY3 families	MODY gene–negative families: Median age at diagnosis <25 years	MODY gene–negative families: Median age at diagnosis ≥25 years	*P* value
Triglycerides (mg/dl)	100 ± 58	154 ± 153	178 ± 140	0.020
Cholesterol (mg/dl)	195 ± 41	206 ± 34	220 ± 45	0.019
HDL cholesterol (mg/dl)	53 ± 14	47 ± 11	44 ± 15	0.17
Hypertension (%)	10.0	33.3	62.5	0.0001
Antihypertensive drugs (%)	3.8	20.9	37.9	0.0001
Nephropathy (%)	18.0	42.9	44.9	0.001
Microalbuminuria (%)	14.8	35.7	38.5	0.008
Overt proteinuria (%)	3.2	7.2	6.4	0.008
Coronary artery disease (%)	17.2	53.3	29.7	0.046
Bypass or angioplasty (%)	3 4	20.0	7.6	0.15
Retinal laser treatment (%)	6.3	16.3	8.5	0.17

Data for quantitative variables are means ± SD. *P* values were calculated after adjusting for differences in sex, age at examination, and time elapsed since diabetes diagnosis. Serum lipid measurements were available for 64, 26, and 154 individuals in the three study groups, respectively. Data for hypertension refer to the total number of individuals for whom complete information on blood pressure values and antihypertensive treatment was available (50, 33, and 136 in the three study groups, respectively). For nephropathy, renal status was evaluated in 245 subjects (61, 28, and 156 in the three study groups, respectively). *P* values were estimated by χ^2 test, with 2 df for the outcome "nephropathy" (microalbuminuria + overt proteinuria) and 4 df for the separate outcomes of microalbuminuria and overt proteinuria. For coronary artery disease, percentages refer to individuals aged >40 years (29, 15, and 118 in the three study groups, respectively).

type of treatment. As far as cardiovascular complications are concerned, no individual younger than age 40 reported a previous diagnosis of ischemic heart disease in all three family groups. Among subjects over this age, a previous diagnosis of angina or heart attack was reported more frequently among MODY gene–negative subjects (29.7 and 53.3% in the two age-at-diagnosis groups, respectively) than among MODY3 individuals (17.2%). This difference in cardiovascular risk was almost statistically significant for the group with age at diagnosis younger than 25 years (OR = 5.9 [0.9–37.5], after adjusting for age at examination, sex, and time since diabetes diagnosis). Similar relative-risk estimates were obtained when a more stringent definition of ischemic heart disease (hospital admission for angioplasty or bypass surgery) was used. No significant difference was observed in the proportions of patients reporting laser treatment for proliferative retinopathy in the three family groups.

CONCLUSIONS — The aim of our study was to define the phenotypic characteristics of early-onset autosomal-dominant type 2 diabetes not accounted for by known MODY genes. To this end, we investigated 39 families that were recently ascertained on the basis of at least two diabetic members diagnosed before age 35 and occurrence of type 2 diabetes in three or more generations. These families had been previously screened for mutations or linkage with MODY genes. In 10 of these kindred, mutations in the HNF-1α gene (MODY3) segregated with diabetes. In the remaining 29, no mutations were found in either the HNF-1α or the HNF-4α (MODY1) genes, and linkage is excluded with the glucokinase (MODY2) locus. Thus, the first finding in this set of families is that forms of early-onset autosomal-dominant type 2 diabetes not linked to known MODY genes not only exist, but are relatively frequent.

The clinical characteristics of diabetes in the 10 families with MODY3 are similar to the original description by Vaxillaire and colleagues (29,30) in French pedigrees and, more recently, by Lehto et al. (18) in four Finnish kindred with MODY3. As previously described, in the majority of cases diabetes is diagnosed before age 35, although age at onset and severity of diabetes vary widely, partially in relation to the location of mutations in the HNF-1α gene (27). Although body weight also appears to influence penetrance and expression of HNF-1α mutations (27), on average obesity is not a characteristic of this type of diabetes. Hyperglycemia in these individuals is related to a defect in the insulin secretion, as testified by the severely impaired insulin response to OGTT. These features are similar to those of diabetes due to HNF-4α mutations (MODY1) (19). A pure pancreatic β-cell defect, although milder, is also described for diabetes due to glucokinase mutations (MODY2) (17).

A quite different picture emerges from the analysis of our 29 families in which early-onset autosomal-dominant diabetes is not linked to known MODY genes. On average, diabetes is diagnosed at an older age among these individuals and is associated with obesity. Most importantly, the insulin response to an oral glucose load is much more heterogeneous than in MODY3 diabetes and is conserved in many individuals, although early insulin secretory defects might have been missed by our OGTT sampling. These features are accompanied by higher serum triglycerides, lower HDL cholesterol levels, and a higher prevalence of hypertension than in the MODY3 families. Taken together, these data suggest that the insulin resistance syndrome—i.e., the clustering of insulin resistance, hypertension, and dyslipidemia (31)—is a frequent feature of early-onset type 2 diabetes not related to known MODY genes. Similar to what is commonly observed for common, later-onset type 2 diabetes (2), the primary defect in some of these forms of diabetes appears to be a decrease in the response of peripheral tissue to insulin, which cannot be fully compensated by an increase of insulin secretion by the β-cell. As previously reported in populations with a high prevalence of type 2 diabetes, such as offspring of diabetic parents, Pima Indians, and Mexican Americans, insulin resistance might occur early in life and precede any evidence of glucose intolerance, whereas the β-cell failure may develop somewhat later, in association with the development of hyperglycemia (32–34). Because of the known effects of hyperglycemia on both insulin secretion and insulin sensitivity, testing this hypothesis will require thorough evaluation of insulin sensitivity (e.g., by means of intravenous glucose tolerance test or glucose clamp) in young family members before they develop diabetes.

Although diabetes is, on average, diagnosed at an older age among MODY gene–negative families than among MODY3 kindred, there is considerable overlap in the age at diagnosis between the two groups. In particular, six of the MODY gene–negative

pedigrees have a median age at diabetes diagnosis younger than 25 years, similar to the MODY3 families. Therefore, these six families, although negative for known MODY genes, fulfill all the criteria for designation as MODY pedigrees. They may correspond to the proportion of MODY cases (~25%) unaccounted for by HNF-1α, HNF-4α, or glucokinase genes in previous studies from France and Great Britain (29,35), although obesity is more common in our families. Interestingly, the characteristics of diabetes in these six "MODY" families are intermediate between the MODY3 pedigrees and the remaining MODY gene–negative families. Serum insulin levels are, on average, higher than among MODY3 individuals both in the fasting state and 2 h after the oral glucose load. A similar pattern is observed for serum fasting C-peptide levels, the insulin requirement among insulin-treated subjects, and risk of hypertension. Thus, it appears that forms of MODY not linked to known MODY genes may also be characterized by some degree of obesity and insulin resistance. Consistent with our findings, a higher insulin response during a full OGTT has been also described by Fajans (36) in two MODY families as compared with the MODY1 RW pedigree.

The finding of ~10% of individuals being positive for β-cell antibodies in each group of families deserves some comments. The presence of antibody-positive subjects among type 2 diabetes individuals has been previously reported, with a prevalence similar to that observed in our families (37). The occurrence of these individuals in type 2 diabetes families has been explained as a chance overlap between autoimmune diabetes and nonautoimmune, familial type 2 diabetes (38). This explanation, however, is contradicted by the fact that antibody-positive subjects were also found among our MODY3 individuals, whose diabetes is known to be not autoimmune, being caused by mutations in the HNF-1α gene. An alternative hypothesis is that the presence of β-cell antibodies, rather than being etiologically related to diabetes, is secondary to the release of β-cell antigens due to β-cell destruction by nonautoimmune mechanisms. Supporting the hypothesis of some degree of β-cell destruction in type 2 diabetes or MODY is the report that subjects with type 2 diabetes can have between 10 and 50% fewer β-cells than control subjects (39).

Another interesting finding is the much higher risk of hypertension, renal complications, and coronary heart disease among the MODY gene–negative families as compared with the MODY3 pedigrees, regardless of the age at examination. These results are even more remarkable if one considers that most of these families are characterized by a shorter time since the diagnosis of diabetes than the MODY3 group. Data on the risk of diabetic complications among subjects with early-onset type 2 diabetes are scant in the literature. One report with which our results can be compared is the one by Velho et al. (30), who described a higher prevalence of renal complications among MODY3 individuals than among subjects with other types of MODY or with common type 2 diabetes. That study, however, included only 26 MODY3 individuals and was limited to the risk of overt proteinuria, rather than also considering incipient nephropathy (microalbuminuria) as was done in our study. More recently, a report from Sweden found a lower prevalence of hypertension and cardiovascular disease in MODY3 than in type 2 diabetes, but no difference in the risk of renal complications (40). The reasons for the higher risk of renal and cardiovascular complications observed in our MODY gene–negative families as compared with MODY3 pedigrees are not clear. It is possible that individuals from the MODY gene–negative families have more sustained hyperglycemia than members of MODY3 families, but this hypothesis seems unlikely. First, among non-insulin-treated individuals, glycated hemoglobin levels are similar in the two groups of families. Second, there are no significant differences in the risk of proliferative retinopathy, which is also strongly affected by the degree of glycemic control (41). Because type 2 diabetes can go undiagnosed for several years, it is also possible that MODY gene–negative individuals, especially older ones, had a longer exposure to hyperglycemia than did MODY3 subjects, in spite of a shorter time since diabetes diagnosis. An alternative hypothesis, however, is that the increased risk of both renal and cardiovascular complications among the MODY gene–negative families may be related to the presence of insulin resistance. Insulin resistance and other features of the so-called insulin resistance syndrome (hypertriglyceridemia, low HDL concentration, and arterial hypertension) are well-recognized risk factors for cardiovascular disease (30). A similar association has been reported in the literature between insulin resistance and microalbuminuria in both type 2 and type 1 diabetes (42,43). Chronic hyperinsulinemia, secondary to insulin resistance, has been suggested as a factor mediating these associations (44,45). Thus, subjects with typical MODY, who secrete little insulin but require only small amounts of exogenous hormone to control their diabetes, may be protected from cardiovascular and renal complications in comparison with subjects with either early- or late-onset type 2 diabetes or with type 1 diabetes. Alternatively, the cellular and metabolic features associated with insulin resistance may be responsible factors, independent of compensatory hyperinsulinemia (46). Finally, one must consider that HNF-1α is expressed in the kidney and that HNF-1α mutations might themselves protect MODY3 subjects from renal complications. As shown in a recent abstract from France (47), MODY3 subjects appear to have a lower tubular re-absorption of glucose, which, by causing a parallel decrease in the tubular re-absorption of sodium, may protect MODY3 patients from hypertension and renal complications.

In conclusion, our findings indicate the existence of forms of early-onset type 2 diabetes that appear to segregate with an autosomal-dominant pattern of inheritance and are distinct from MODY. This type of diabetes is relatively frequent and is characterized by a high prevalence of obesity, insulin resistance, hypertension, dyslipidemia, and renal and cardiovascular complications. Because of the simple Mendelian pattern of inheritance and the availability of large families, the goal of identifying the genes involved in these forms of diabetes should be particularly feasible, as was the case for MODY. The fact that insulin resistance is a common feature of early-onset type 2 diabetes makes it likely that some of these genes may also contribute to later-onset type 2 diabetes.

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References

1. Warram JH, Rich SS, Krolewski AS: Epidemiology and genetics of diabetes melli-

tus. In *Joslin's Diabetes Mellitus* 13th ed. Kahn CR, Weir G, Eds. Philadelphia, Lea & Febiger, 1994, p. 201–215
2. Kahn CR: Insulin action, diabetogenes, and the cause of type II diabetes. *Diabetes* 43:1066–1084, 1994
3. Kahn CR, Vicent D, Doria A: Genetics of non-insulin-dependent (type II) diabetes mellitus. *Ann Rev Med* 47:509–531, 1996
4. Tattersall RB, Fajans SS: A difference between the inheritance of classical juvenile-onset and maturity-onset type diabetes of young people. *Diabetes* 24:44–53, 1974
5. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Thanh T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Brostow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick M: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266:66–71, 1994
6. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G: Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378:789–792, 1995
7. Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clement K, Fougerousse F, Tanizawa Y, Weissenbach J, Beckmann JS, Lathrop GM, Passa P, Permutt MA, Cohen D: Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:162–164, 1992
8. Hattersley AT, Turner RC, Permutt MA, Patel P, Tanizawa Y, Chiu KC, O'Rahilly S, Watkins PJ, Wainscoat JS: Linkage of type 2 diabetes to the glucokinase gene. *Lancet* 339:1307–1310, 1992
9. Vionnet N, Stoffel M, Takeda J, Yasuda K, Bell GI, Zouali H, Lesage S, Velho G, Iris F, Passa P, Froguel P, Cohen D: Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:721–722, 1992
10. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Turner RC, Velho G, Chevre JC, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young. *Nature* 384:455–458, 1996
11. Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young. *Nature* 384:458–460, 1996
12. Stoffers DA, Ferrer J, Clarke WL, Habener JF: Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 17:138–139, 1997
13. Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI: Mutation in hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
14. Yamada S, Nishigori H, Onda H, Takahashi K, Kitano N, Morikawa A, Takeuchi T, Takeda J: Mutations in the hepatocyte nuclear factor-1alpha gene (MODY3) are not a major cause of late-onset NIDDM in Japanese subjects. *Diabetes* 46:1512–1513, 1997
15. Urhammer SA, Rasmussen SK, Kaisaki PJ, Oda N, Yamagata K, Moller AM, Fridberg M, Hansen L, Hansen T, Bell GI, Pedersen O: Genetic variation in the hepatocyte nuclear factor-1 alpha gene in Danish Caucasians with late-onset NIDDM. *Diabetologia* 40:473–475, 1997
16. Moller AM, Urhammer SA, Dalgaard LT, Reneland R, Berglund L, Hansen T, Clausen JO, Lithell H, Pedersen O: Studies of the genetic variability of the coding region of the hepatocyte nuclear factor-4 alpha in Caucasians with maturity onset NIDDM. *Diabetologia* 40:980–983, 1997
17. Velho G, Froguel P, Clement K, Pueyo ME, Rakotoambinina B, Zouali H, Passa P, Cohen D, Robert JJ: Primary pancreatic beta-cell secretory defect caused by mutations in glucokinase gene in kindreds of maturity onset diabetes of the young. *Lancet* 340:444–448, 1992
18. Lehto M, Tuomi T, Mahtani MM, Widen E, Forsblom C, Sarelin L, Gullstrom M, Isomaa B, Lehtovirta M, Hyrkko A, Kanninen T, Orho M, Manley S, Turner RC, Brettin T, Kirby A, Thomas J, Duyk G, Lander E, Taskinen MR, Groop L: Characterization of the MODY3 phenotype: early-onset diabetes caused by an insulin secretion defect. *J Clin Invest* 99:582–591, 1997
19. Herman WH, Fajans SS, Ortiz FJ, Smith MJ, Sturis J, Bell GI, Polonsky KS, Halter JB: Abnormal insulin secretion, not insulin resistance, is the genetic or primary defect of MODY in the RW pedigree. *Diabetes* 43:40–46, 1994
20. O'Rahilly S, Spivey RS, Holman RR, Nugent Z, Clark A, Turner RC: Type II diabetes of early onset: a distinct clinical and genetic syndrome? *Br Med J* 294:923–928, 1987
21. Vague P, Lassmann V, Grosset C, Vialettes B: Type II diabetes in young subjects: a study of 90 unrelated cases. *Diabete Metab* 13:92–98, 1987
22. O'Rahilly S, Turner RC: Early-onset type 2 diabetes vs maturity-onset diabetes of the youth: evidence for the existence of two discrete diabetic syndromes. *Diabet Med* 5:224–229, 1988
23. Harris MI: Impaired glucose tolerance in the U.S. population. *Diabetes Care* 12:464–474, 1989
24. Krolewski AS, Laffel LMB, Krolewski M, Quinn M, Warram JH: Glycated hemoglobin and risk of microalbuminuria in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 332:1252–1255, 1995
25. Grubin CE, Daniels T, Toivola B, Landin-Olsson M, Hagopian WA, Li L, Karlsen AE, Boel E, Michelsen B, Lernmark A: A novel radioligand binding assay to determine diagnostic accuracy of isoform-specific glutamic acid decarboxylase antibodies in childhood IDDM. *Diabetologia* 37:344–350, 1994
26. Christie MR, Vohra G, Champagne D, Delovitch TL: Distinct antibody specificities to a 64-kD islet cell antigen in type I diabetes as revealed by trypsin treatment. *J Exp Med* 172:789–794, 1990
27. Yang Y, Antonellis A, Malecki M, Wantman M, Warram JH, Krolewski AS: Mutation screening of the HNF-1α gene in North American MODY families (Abstract). *Am J Hum Genet* 61 (Suppl.):A352, 1997
28. Rothman KJ: *Modern Epidemiology* Boston, MA, Little, Brown & Company, 1986
29. Vaxillaire M, Boccio V, Philippi A, Vigouroux C, Terwilliger J, Passa P, Beckmann JS, Velho G, Lathrop GM, Froguel P: A gene for maturity onset diabetes of the young maps to chromosome 12q. *Nat Genet* 9:418–423, 1995
30. Velho G, Vaxillaire M, Boccio V, Charpentier G, Froguel P: Diabetes complications in NIDDM kindreds linked to the MODY3 locus on chromosome 12 q. *Diabetes Care* 19:915–919, 1996
31. Reaven GM: Banting Lecture 1988: role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1988
32. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin-resistance in the development of type II diabetes mellitus: results of a 25-years follow-up study. *Lancet* 340:925–929, 1992
33. Gulli G, Ferrannini E, Stern M, Haffner S, DeFronzo RA: The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. *Diabetes* 41:1575–1586, 1992
34. Knowler WC, Pettitt DJ, Saad MF, Bennett PH: Diabetes mellitus in Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 6:1–27, 1990
35. Frayling TM, Bulamn MP, Ellard S, Appleton M, Dronsfield MJ, Mackie AD, Baird JD,

Kaisaki PJ, Yamagata K, Bell GI, Bain SC, Hattersley AT: Mutations in the hepatocyte nuclear factor-1alpha gene are a common cause of maturity-onset diabetes of the young in the U.K. *Diabetes* 46:720–725, 1997
36. Fajans SS: Heterogeneity of insulin secretion in type II diabetes. *Diabetes Metab Rev* 2:347–361, 1986
37. Turner R, Stratton I, Horton V, Manley S, Zimmet P, Mackay IR, Shattock M, Bottazzo GF, Holman R: UKPDS 25:autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. *Lancet* 350:1288–1293, 1997
38. Elbein SC, Wegner K, Miles C, Yu L, Eisenbarth G: The role of late-onset autoimmune diabetes in white familial NIDDM pedigrees. *Diabetes Care* 20:1248–1251, 1997
39. Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJ, Holman RR, Turner RC: Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res* 9:151–159, 1988
40. Isomaa B, Henricsson, Lehto M, Forsblom C, Karanko S, Sarelin L, Haggblom M, Groop L: Chronic diabetic complications in patients with MODY3 diabetes. *Diabetologia* 41:467–473, 1998
41. DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
42. Groop L, Ekstrand A, Forsblom C, Widen E, Groop PH, Teppo AM, Eriksson J: Insulin resistance, hypertension and microalbuminuria in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:642–647, 1993
43. Yip J, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti GC: Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. *Lancet* 342:883–887, 1993
44. Pyorala K: Relationship of glucose tolerance and plasma insulin to the incidence of coronary artery disease: results from two population studies in Finland. *Diabetes Care* 2:131–141, 1979
45. Ducimetiere P, Eschwege E, Papoz L, Richard JL, Claude JR, Rosselin G: Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary artery disease mortality in a middle-aged population. *Diabetologia* 19:205–210, 1980
46. Stern MP: Diabetes and cardiovascular disease: the "common soil" hypothesis. *Diabetes* 44:369–374, 1995
47. Velho G, Benque-Blanchet F, Vaxillaire M, Pontoglio, M, Froguel P, Friedlander G, Prie' D: Renal proximal tubular defects associated to the MODY3 phenotype (Abstract). *Diabetologia* 41 (Suppl. 1):A108, 1998