Characterization of the MODY3 Phenotype

Early-Onset Diabetes Caused by an Insulin Secretion Defect

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Abstract

Maturity-onset diabetes of the young (MODY) type 3 is a dominantly inherited form of diabetes, which is often misdiagnosed as non–insulin-dependent diabetes mellitus (NIDDM) or insulin-dependent diabetes mellitus (IDDM). Phenotypic analysis of members from four large Finnish MODY3 kindreds (linked to chromosome 12q with a maximum lod score of 15) revealed a severe impairment in insulin secretion, which was present also in those normoglycemic family members who had inherited the MODY3 gene. In contrast to patients with NIDDM, MODY3 patients did not show any features of the insulin resistance syndrome. They could be discriminated from patients with IDDM by lack of glutamic acid decarboxylase antibodies (GAD-Ab). Taken together with our recent findings of linkage between this region on chromosome 12 and an insulin-deficient form of NIDDM (NIDDM2), the data suggest that mutations at the MODY3/NIDDM2 gene(s) result in a reduced insulin secretory response, that subsequently progresses to diabetes and underlines the importance of subphenotypic classification in studies of diabetes. (*J. Clin. Invest.* 1997. 99:582–591.) Key words: maturity-onset diabetes of the young • non–insulin-dependent diabetes mellitus • insulin deficiency • insulin sensitivity • genetics

Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is a heterogeneous disorder with a complex pattern of inheritance; it generally becomes manifest after 40 yr of age and is characterized by both impaired β cell function and insulin resistance (1–3). The relative contribution of these two defects to the manifestation of diabetes is still a matter of debate (4), but may vary among populations. While insulin resistance seems to predominate in some populations (i.e., Native American–derived) (5, 6), impaired β cell function may be more prevalent in others (i.e., Scandinavian) (2, 7). Part of this heterogeneity could be explained by admixture of different diabetic subtypes.

In contrast to adult-onset NIDDM, maturity-onset diabetes of the young (MODY) shows clear dominant inheritance, early onset ($\sim$ 25 yr) and high penetrance (8). MODY is thought to account for $<$ 5% of all cases with diabetes. However, its true prevalence is unknown due to the lack of distinguishing phenotypes and cloned genetic loci for most of the MODY subtypes.

MODY itself is heterogeneous. Three distinct forms of MODY have been genetically mapped, and based upon the existence of MODY families unlinked to any of these chromosome regions, other MODY genes remain to be identified. The rare MODY1 has been linked to a region near the adenosine deaminase (ADA) locus on chromosome 20 in a single large family (9). No gene has been identified yet due to the small number of MODY1 cases described. The mild MODY2 phenotype is one of hyperglycemia rather than diabetes per se and can be caused by several mutations in the glucokinase gene (GK) on chromosome 7 (10). Glucokinase is essential for glucose metabolism in the β cell; some mutations in the glucokinase gene reduce the affinity of the enzyme for glucose resulting in a small increase in the plasma glucose concentrations (11). While this subtype accounts for 60% of MODY in France (12), it is less prevalent elsewhere (13, 14). Recently, MODY3 was identified by linkage to a region on the long arm of chromosome 12 (15). Other than its chromosome 12 linkage, little

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Received for publication 20 September 1996 and accepted in revised form 6 December 1996.

J. Clin. Invest.

0021-9738/97/02/0582/10 $2.00
Volume 99, Number 4, February 1997, 582–591

1. *Abbreviations used in this paper:* BMI, body mass index; CV, coefficient of variation; FBG, fasting blood glucose; GAD-Ab, glutamic acid decarboxylase antibodies; IDDM, insulin-dependent diabetes mellitus; IVGTT, intravenous glucose tolerance test; MODY, maturity-onset diabetes of the young; NIDDM, non–insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; PI, proinsulin; WHO, World Health Organization.

clinical phenotypic information has been available to distinguish MODY3 from NIDDM or insulin-dependent diabetes mellitus (IDDM).

With the goal of carrying out genetic studies of NIDDM, we established the Botnia study in 1990 to identify multiplex families with diabetes in an isolated population on the western coast of Finland (near the Gulf of Botnia) (16). About 5,000 individuals, including 1,230 patients with diabetes from 600 families, have been studied to date. From this family collection, we identified four large multigenerational families with early-onset diabetes linked to chromosome 12 (MODY3). Because of the unusually large size of these MODY3 families, and our early observation that the phenotype in these families differed from that in common adult-onset NIDDM, we set out to characterize in detail the clinical and metabolic phenotype of MODY3, and to phenotypically distinguish MODY3 from NIDDM and IDDM. Further, since we recently mapped a form of adult-onset NIDDM (NIDDM2; also associated with insulin deficiency but with an average age at onset of 58 yr) to precisely this region of chromosome 12 (17), this study may help to establish whether the defects in MODY3 and NIDDM2 are caused by different alleles of the same gene.

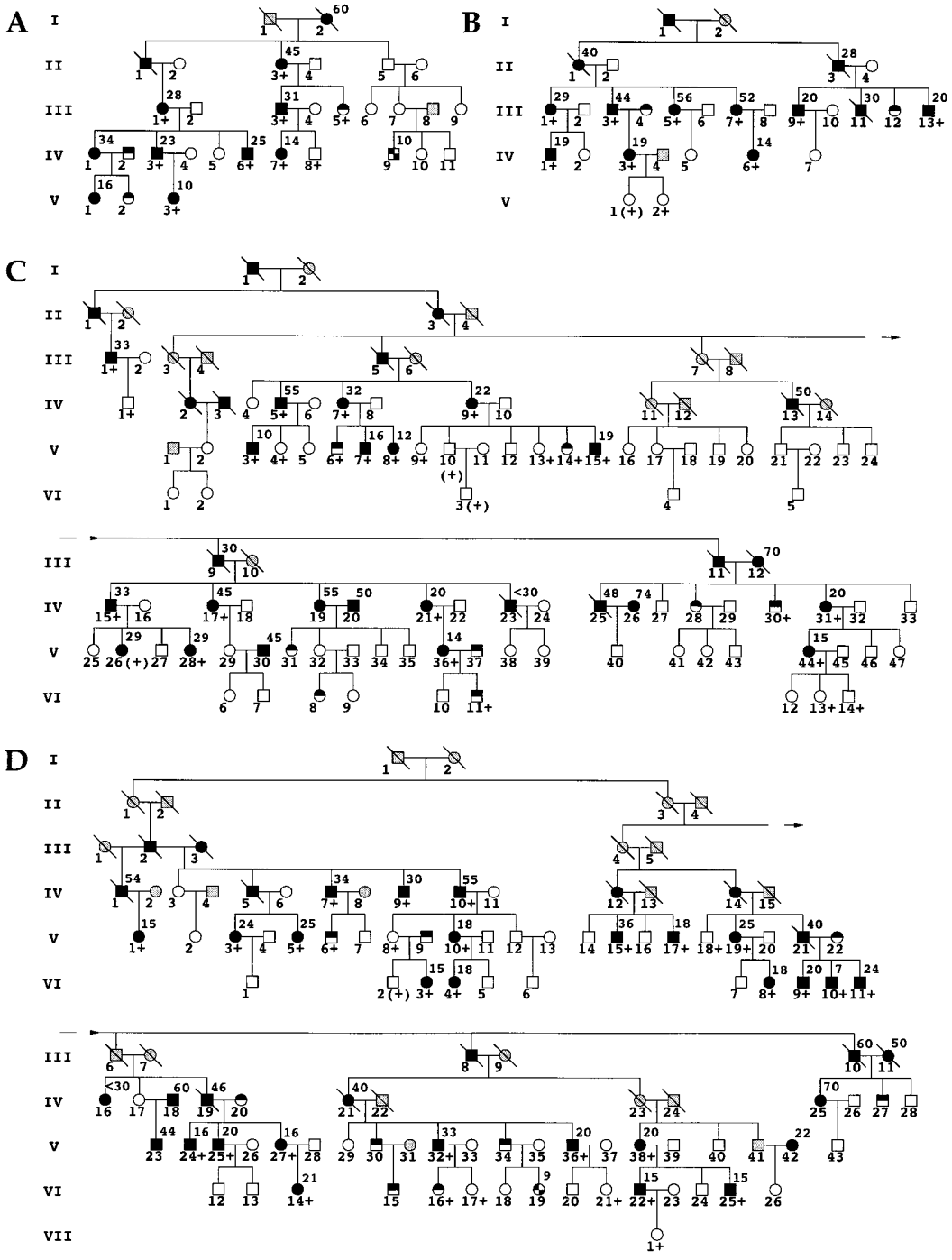

Figure 1. Pedigrees of four Finnish MODY3-linked families. Diabetic patients are indicated by closed symbols, nondiabetic subjects by open symbols, and undiagnosed subjects by gray symbols. Subjects with impaired glucose tolerance are indicated by half-filled symbols. In pedigrees A and D, IDDM subjects are indicated by checkered symbols. MODY3-linked individuals are indicated by plus signs. Age at onset of diabetes is shown at the upper right corner of the symbol. The Roman numerals depict generations. In the text, the subjects are referred to by a code consisting of generation and individual number, e.g., IV-7.

Methods

Subjects. NIDDM families were studied from the Botnia region in Western Finland (16). In four large families diabetes (defined according to World Health Organization [WHO] criteria) was expressed at early age (at least two patients with onset younger than 25 yr) and transmitted in an autosomal dominant fashion fulfilling the criteria for MODY (Fig. 1). Family A was ascertained initially as a relatively small pedigree that was later extended to include more distantly related family members once the preliminary analysis provided support for linkage to chromosome 12. Most diabetic patients in pedigree A (II-3, III-1, III-3, IV-3, IV-6, IV-7, and V-3) shared a common phenotypic profile which consisted of (*a*) low fasting insulin (4.1±1.1 mU/liter) and C-peptide (0.35±0.11 nmol/liter) levels and low insulin (incremental area under curve 1049±537 mU/liter) and C-peptide (incremental area under curve 27.6±12.5 nmol/liter) responses to oral glucose tolerance test (OGTT); (*b*) normal body mass index (BMI; 22.8±3.8 kg/m^2); and (*c*) lack of glutamic acid decarboxylase antibodies (GAD-Ab). However, as the family was extended, a few diabetic patients (IV-1, V-1, and IV-9) were identified that did not fit this clinical profile. Specifically, patients IV-1 and V-1 were obese (BMI 28.3 and 33.9 kg/m^2, respectively) and had high fasting insulin (18.8 and 40.8 mU/liter) and C-peptide levels (1.14 and 1.29 nmol/liter). Patient IV-9, who was insulin-treated since diagnosis, was lean (BMI 18.6 kg/m^2) and displayed high titers of GAD antibodies and undetectable C-peptide levels. We considered patients IV-1 and V-1 to represent common NIDDM and patient IV-9 to have IDDM. When these three patients were genotyped, they were found not to share the copy of chromosome 12 coinherited by the seven other diabetics in family A.

Families B, C, and D were ascertained ~ 1 yr after family A (Fig. 1). These families were initially identified as containing several diabetic patients with young age at onset, and similarly extended. Families B, C, and D all originated from an area of Botnia geographically distinct from that of family A.

In total, we examined 230 (74 diabetic and 156 nondiabetic) subjects from four families. Since no phenotypic heterogeneity was detected between the MODY3 families with respect to age at onset, BMI, glucose, and insulin values, the families were analyzed together. The clinical profile of the 57 patients (MODY3 patients) who had inherited the MODY3-chromosome (See Results, MODY3 haplotype and segregation) was compared with that of the 12 patients not inheriting the MODY3-chromosome (NIDDM patients). The two GAD-Ab positive subjects (A; IV-9 and D; VI-19) considered to have IDDM were excluded from the analysis. Since the group of NIDDM patients was relatively small, we also compared the MODY3 phenotype in these families with that of 1,068 NIDDM patients from the Botnia Study (Table I, *Botnia NIDDM*). Similarly, we compared nondiabetic individuals with the MODY3 chromosome (*MODY3 carriers*; $n = 18$) with nondiabetic family members not inheriting this copy of chromosome 12 (*Family controls*; $n = 138$) and age-matched controls from the Botnia study (*Botnia controls*; $n = 92$) (Table I).

Measurements. A standard (75 gram) OGTT was performed after 12 h of overnight fasting. For subjects < 12 yr of age or with fasting blood glucose (FBG) > 10 mmol/liter, only fasting samples were analyzed. For the MODY3 families OGTT was performed for 158 of 227 family members. Blood samples for the determination of blood glucose, serum insulin, and C-peptide were drawn at −10, 0, 30, 60, and 120 min; and for plasma proinsulin (PI) −10, 0, and 120 min. Fasting blood samples were also drawn for the measurements of GAD-Ab, glycohemoglobin (HbA_{1c}), serum total cholesterol, HDL2 and HDL3

Table I. Clinical Characteristics of Diabetic and Nondiabetic Subjects

	Diabetic patients					Nondiabetic patients				
	Botnia NIDDM	*P* value	MODY3 patients	*P* value	NIDDM patients	Botnia controls	*P* value	MODY3 carriers	*P* value	Family controls
Subjects (M/F)	1068 (466/602)		57 (27/30)		12 (5/7)	92 (42/50)		18 (8/10)		138 (68/70)
Age (yr)	69±11		43±17	0.012	57±18	33±7		29±14	0.001	43±16
Age at onset (yr)	60±12	0.001	26±12	0.001	50±18	—		—		—
Duration (yr)	8.1±7.2	< 0.0001	18.0±12.3	< 0.0001	8.6±9.7	—		—		—
BMI (kg/m^2)	28.1±4.7	< 0.001	24.8±4.2	< 0.001	29.0±4.1	23.9±3.2	0.001	20.9±3.8	0.001	25.8±4.6
Fat mass (%)	0.30±0.07		0.26±0.08		0.34±0.05	0.25±0.07		0.23±0.07	0.02	0.28±0.07
WHR (Males)	0.98±0.06		0.96±0.07		1.01±0.01	0.91±0.06		0.95±0.07		0.99±0.04
(Females)	0.88±0.07		0.86±0.07		0.95±0.09	0.81±0.05		0.85±0.05		0.85±0.07
HbA_{1c} (%)	8.9±2.0		8.0±1.6		8.5±2.0	5.2±0.4	0.007	5.5±0.3		5.5±0.5
FBG (mmol/l)	8.6±2.8	< 0.005*	8.4±2.7		8.6±2.9	4.8±0.4	< 0.004*	4.4±0.5	< 0.004*	4.8±0.5
2h-Glucose (mmol/l)	14.4±5.1		13.3±3.9		15.2±6.8	5.0±0.9	0.0001	6.5±1.4	0.0001	5.5±1.4
Glucose area	623±225		598±174		643±198	109±98	0.001	307±121	0.001	203±144
FS-Insulin (mU/liter)	16.2±13.6	< 0.001*	11.2±19.9	< 0.001*	22.8±16.1	6.6±3.3	< 0.003*	4.7±2.1	< 0.003*	7.9±6.0
Insulin area	5110±4400	< 0.002*	1330±900	< 0.002*	4670±3270	4014±2432	< 0.0001*	2010±880	< 0.0001	5270±4810
FS-C-Peptide (nmol/liter)	0.65±0.41		0.36±0.16	< 0.01*	1.06±0.47	0.31±0.16	< 0.01*	0.39±0.17	< 0.01*	0.57±0.29
Cholesterol (mmol/liter)	5.97±1.26	< 0.05	5.30±1.12		6.01±1.14	4.80±0.87		4.79±0.88		5.24±1.15
S-TRIGL (mmol/liter)	2.04±1.41	< 0.02*	1.26±0.64		1.74±0.66	1.00±0.52		0.92±0.30		1.17±0.79
HDL-CHOL (mmol/liter)	1.24±0.33		1.44±0.28		1.21±0.28	1.48±0.31		1.47±0.25		1.40±0.34
HDL2-CHOL (mmol/liter)	0.42±0.23		0.50±0.22		0.34±0.10	0.60±0.25		0.52±0.18		0.45±0.23
HDL3-CHOL (mmol/liter)	0.82±0.17		0.94±0.15		0.87±0.20	0.88±0.17		0.94±0.11		0.96±0.17
Systolic BP (mmHg)	149±21		129±16		142±23	118±11		120±14		130±17
Diastolic BP (mmHg)	82±11		79±9		79±12	72±8		73±9		79±9
GAD-Ab positives (*n*)	98 (10%)		0		0	4 (4.3%)		0		1 (0.7%)

Results are given as uncorrected values with mean±SD. Analysis of variance with Fisher LSD test for multiple comparisons, using age, BMI, and duration of diabetes as covariates, was used for the statistical comparison. *Values were logarithmically transformed for statistics. WHR, waist-to-hip ratio.

cholesterol, and triglyceride concentration. Fat mass was measured with infrared spectroscopy from the outer layer of the biceps on the dominant arm using a Futrex 5000 device (Futrex Inc., Gaithersburg, MD). Body weight and height were measured with subjects in light clothing without shoes. In determination of waist-to-hip ratio, waist circumference was measured with a soft tape on standing subjects midway between the lowest rib and the iliac crest, and hip circumference was measured over the widest part of the gluteal region. Three blood pressure recordings were obtained after 30 min of rest at 5-min intervals from the right arm of a sitting person. The coefficients of variation (CV) of the three systolic and diastolic blood pressure measurements were 4 and 5.9%, respectively.

Measurements of insulin secretion and insulin sensitivity. Insulin secretion was also measured in response to an intravenous glucose tolerance test (IVGTT) and insulin sensitivity during a euglycemic hyperinsulinemic clamp. Moreover, to obtain independent measurements of insulin secretion and insulin sensitivity during the same test, we performed these two tests in a sequential manner during the same day in a subset of subjects (Botnia clamp). In brief, 0.3 g/kg body weight of a 50% glucose solution was given intravenously at time 0. After 60 min, an infusion (infusion rate of 45 mU/m^2) of short-acting human insulin (Actrapid; Novo Nordisk, Gentofte, Denmark) was started and continued until 180 min. Blood samples for the measurement of blood glucose were obtained at −10, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 min after which they were obtained at 5-min intervals throughout the euglycemic clamp. A variable infusion of 20% glucose was started to maintain the blood glucose concentration unchanged at 5.5 mmol/liter with a CV of 6%. Blood samples for the measurement of insulin were drawn at −10, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 120, 170, and 180 min. The incremental trapezoidal area during the first 10 min of the test was called the first phase insulin secretion, and during the 10–60 min period it was called the late phase insulin secretion. Insulin sensitivity was calculated from the rate of glucose infusion during the last 60 min of the euglycemic clamp and expressed per kilogram of lean body mass. In 10 patients we performed paired tests comparing the Botnia clamp with a similar euglycemic clamp without prior intravenous injection of glucose. The two tests correlated strongly ($r = 0.94$; $P = 0.0001$), although the Botnia clamp gave on average 7% higher rates of glucose infusion than the euglycemic clamp.

Assays. Blood glucose during the OGTT was measured on duplicates with a hexokinase method with a CV of < 1% (Boehringer Mannheim, Mannheim, Germany). Plasma glucose during the euglycemic clamp was measured with a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Serum insulin concentrations were measured with a double antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) with an interassay CV of 5%. Plasma C-peptide concentrations were measured in duplicates by radioimmunoassay with an interassay CV of 9% (18). Plasma PI concentrations were measured in duplicates by ELISA using murine monoclonal antibodies, PEP-001 and HUI-001 (Novo Nordisk, Bagsvaerd, Denmark), with an interassay CV of 12% at 6.9 pmol/liter, 8% at 16.8 pmol/liter, and 6.7% at 63.2 pmol/liter. The ELISA cross-reacts with split 32-33 PI, des (32, 33) PI, and split 65-66 PI. GAD-Ab were determined by a modified radiobinding assay using ^{35}S-labeled recombinant human GAD65 (19, 20). HbA_{1c} concentrations were measured by high pressure liquid chromatography. The reference values for the assay were 5–7%. Serum total cholesterol, HDL subfractions (after precipitation), and triglyceride concentrations were measured on a Cobas Mira analyzer (Hoffman LaRoche, Basel, Switzerland).

PCR and gel conditions. Four families were tested for three known MODY candidate regions: 18 polymorphic markers on chromosome 12 (Fig. 2), one marker on chromosome 20 (ADA locus) (21), and two markers on chromosome 7 (glucokinase gene) (22, 23). For haplotype analysis of the MODY3 chromosomes, eight additional markers were genotyped on chromosome 12 (Table II). The PCR were carried out in a volume of 20 μl containing 25 ng of genomic DNA (isolated from peripheral blood lymphocytes), 16 mM ammoniumsulphate, 67 mM Tris-HCl (pH 8.8), 0.01% Tween 20, 1.5 mM $MgCl_2$, 200 μM dNTP, 3 pmol of phosphorylated ([^{32}P]γ-dATP; Amersham International, Buckinghamshire, United Kingdom) primer I, 3 pmol of primer II, and 0.5 U AmpliTaq DNA-polymerase (Perkin Elmer, Branchburg, NJ). PCR was initiated with denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. PCR products were mixed 1:1 with sample buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) before denaturing gel electrophoresis (5% acrylamide/bisacrylamide 19:1, 7 M urea, 1× TBE).

Linkage analysis. Given the evidence for multiple types of diabetes in family A we wanted to refine the MODY3 phenotype for the purpose of linkage analysis. Based on the phenotype of MODY3 patients in family A we predicted the phenotype in families B, C, and D. Thus, individuals were considered affected if they had both diabetes (as defined by the WHO criteria) and low stimulated insulin

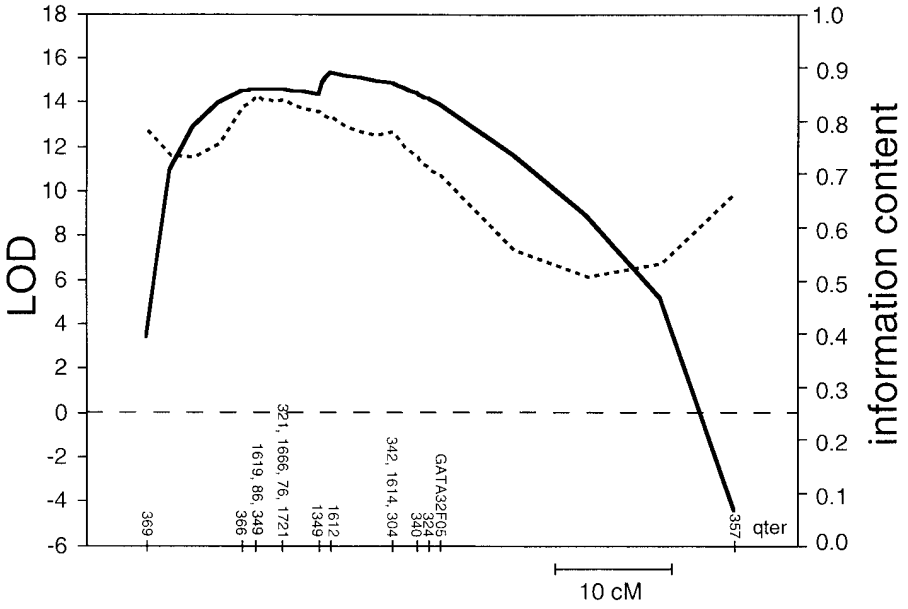

Figure 2. Linkage analysis of NIDDM associated with low stimulated insulin levels (see Methods) in four MODY families. Markers are shown with the prefix "D12S" omitted. LOD scores and information content were calculated using the GENEHUNTER computer package, shown as solid and dotted lines, respectively.

Table II. Comparison of MODY3 Haplotypes between Four MODY3 Families

Genetic distance (cM)	Marker	Family A	B	C	D
	D12S366	K	N	K	M
1					
	D12S86	J	K	S	K
	D12S349	I	E	D	E
	D12S1619	F	F	C	F
2					
	D12S76	E	F	D	F
	D12S321	A	D	C	D
	D12S395	F	B	F	B
	D12S1666	A	B	D	B
	D12S1721*	B	C	C	C
	PLA2*	F	F	F	F
2					
	GATA32A10	E	D	D	D
	GGAT1E2	B	E	E	E
	D12S378	E	B	B	B
	D12S807	D	G	G	G
	D12S820	D	C	C	C
	D12S837	C	F	F	F
	D12S1349	I	B	B	B
	D12S1612	V	M	M	M
5					
	D12S304	D	E	E	D
	D12S342	U	U	U	T
	D12S1614	B	F	F	A
2					
	D12S340	E	E	E	E
1					
	D12S324	B	B	B	—

Locus order was inferred from published maps (29–33) and positions of recombination in MODY3 families. Markers that were unordered relative to one another are grouped together. Markers GATA32A10 and GGAT1E2 are derived from the Cooperative Human Linkage Center (CHLC) and have not yet been assigned locus number. *Markers ordered according to shared alleles between families B, C, and D.

levels (< 25 mU/liter at the 30 min time point during the OGTT). Subjects with either a history of diabetes or impaired glucose tolerance, or who were young (< 35 yr of age at OGTT) were considered of unknown affectation. Of the remaining subjects, those with normal glucose tolerance were considered unaffected.

We used a linkage model with disease allele frequency of 0.01, and assuming autosomal dominant inheritance, with equal female and male recombination rates. The penetrances for homozygous normal, heterozygous, and homozygous MODY3 were 0.003, 0.90, and 0.90, respectively, giving a phenocopy rate among those affected of ~ 1.7%. In a separate test for linkage of NIDDM only (as defined by the WHO), the penetrance of the homozygous normal was raised to 0.03, increasing the phenocopy rate to ~ 17%. Allele frequencies used were calculated based on the originals in the four large families (n = 108 individuals), together with 20 unrelated normoglycemic Botnia controls, genotyped as part of an earlier study (total n = 256 chromosomes) (17).

Linkage to the MODY1 and MODY2 regions was tested using the marker ADA (known to be linked to MODY1) and two markers in the MODY2 glucokinase gene (GCK-A and GCK-P), respectively. The genetic map of the MODY3 region was based on a subset of 17 markers from the Genethon map and one CHLC marker (GATA32F05). The Genethon loci were ordered at odds of > 1,000:1 (with the exception of the three sets of loci shown in Fig. 2, which are genetically inseparable; and the markers D12S1349 and D12S1612, which are 1 cM apart, and whose order may be inverted). The genetic distances (in cM) used in the MODY3 map are those published: D12S369 - (8) - D12S366 - (1) - D12S1619 / D12S86 / D12S349 - (2) - D12S321 / D12S1666 / D12S76 / D12S1721 - (3) - D12S1349 - (1) - D12S1612 - (5) - D12S1614 / D12S304 / D12S342 - (2) - D12S340 - (1) - D12S324 - (1) - GATA32F05 - (23) - D12S357. This marker order is consistent with our own radiation hybrid mapping of these loci (data not shown) and with the order used in our earlier study of a distinct panel of Botnia families (17).

Multipoint linkage analysis of the MODY3 phenotype to each of the three known MODY regions was performed using the GENEHUNTER program (24). We selected GENEHUNTER due to its capacity to analyze inheritance information at many loci simultaneously. (Nonetheless, two- or three-point lod scores obtained with the FASTLINK program were similar to those calculated using GENEHUNTER.) To accommodate the pedigree size limitations of GENEHUNTER, pedigrees C and D were divided into nonoverlapping branches that could be analyzed separately. Pedigree C was divided into three branches of two, five, and six affected individuals each, and pedigree D was divided into four branches of two, four, seven, and nine affected individuals each. For both families, scores for each branch were summed to find the total LOD score of each pedigree. Because dividing the pedigrees into branches results in some loss of power, the family totals will underestimate the true LOD scores of both pedigrees.

GENEHUNTER was also used to haplotype individuals in the four pedigrees across the MODY3 region. For each pedigree, GENEHUNTER calculates the probability distribution over all possible inheritance vectors for a set of markers, then assigns haplotypes to individuals based upon observed genotypes and maximum likelihood estimates of missing information. Families were again divided into branches that could be analyzed separately.

Statistical analysis. Data are expressed as mean±SD or SEM. Since insulin and PI concentrations were not normally distributed, in the text we have also presented data for insulin and PI as medians [interquartile range (75–25%)]. Differences between groups were tested with analysis of covariance with age, BMI, and duration of diabetes as covariates using a SOLO statistical package (Biomedical Data Processing, Los Angeles, CA). To adjust for multiple comparisons when ANOVA showed a significant difference between groups ($P < 0.05$), Fisher LSD post-hoc test was used to identify which group differences accounted for the significant P value. The P value shown is the smallest one with which all group differences detected at 0.05 level were still present.

Results

Linkage analysis and heterogeneity in MODY3 families. No linkage was detected to either MODY1 or MODY2, as evidenced by negative lod scores between the diabetic phenotype (as described above) and each of the markers tested (data not shown). Genotypic data were analyzed for 18 markers throughout the MODY3 region on chromosome 12q. We obtained highly significant support for linkage to MODY3 when all four families were analyzed together. The lod score throughout the region D12S366-GATA32F05 was consistently high (Z > 14), and maximal at marker D12S1612 (Z = 15.29, Fig. 2). When NIDDM was used as a phenotype, the maximal lod score was slightly lower (Z = 13.10 at D12S1612; see Methods). The individual maximum likelihood family lod scores

were Z = 1.98, 2.55, 3.49, and 7.46 for families A, B, C, and D, respectively.

MODY3 haplotype and penetrance. The three families (B, C, and D) that originated from the same region of Botnia shared a conserved haplotype carrying 10 identical alleles across the MODY3 region supporting a role for a common mutation in these families (Table II). The chromosome 12 haplotype shared in family A (Table II) differed from the conserved haplotype shared by the other families suggesting segregation of a MODY3 allele of distinct mutational origin. These two chromosome 12 haplotypes are hereafter referred to as the MODY3-chromosome. As there were no obvious phenotypic differences between MODY3 patients from family A and those from families B, C, and D (see below), the four families were pooled to analyze penetrance.

The large size of this data set (57 affected individuals in four families) makes it useful for estimating the penetrance of MODY3. The phenotype defined in this study (NIDDM associated with impaired insulin secretion) had a penetrance of 76% by age 30–40 yr among individuals carrying the MODY3-chromosome, and did not substantially increase with age. With a broader phenotype (17), including as affected individuals, those with either a history of diabetes or an impaired glucose tolerance, the penetrance was slightly higher (85%).

Clinical characteristics of MODY3 patients. To extend the families, we screened all primary relatives of MODY3 patients by OGTT. In this screening process, we diagnosed 12 diabetic patients of whom 8 had inherited the MODY3-chromosome. Thus, 31% of MODY3 carriers were diagnosed with diabetes at screening, compared with 3% of the noncarriers. MODY3 patients had clearly lower age at onset of diabetes than NIDDM patients (26±12 vs. 57±18 yr; $P = 0.001$) (Table I). However, in 26% of them diabetes was diagnosed after 30 yr of age. Of 29 diabetic women with the MODY3-chromosome, 11 (38%) have had gestational diabetes during pregnancy compared with none of the diabetic women ($n = 7$) without the MODY3-chromosome. Despite the marked difference in age at onset of diabetes, mode of treatment did not differ between the MODY3 patients and the Botnia NIDDM patients: diet (35 vs. 44%), oral hyperglycemic agents (25 vs. 23%), and insulin (40 vs. 33%).

The MODY3 patients had a lower BMI ($P < 0.001$) than NIDDM patients from the same families. Glycemic control as judged from FBG and HbA_{1c} concentrations did not significantly differ between the MODY3 patients, NIDDM patients, and Botnia NIDDM patients. None of the MODY3 patients was GAD-Ab positive compared with 10% (98/1001) of the Botnia NIDDM patients.

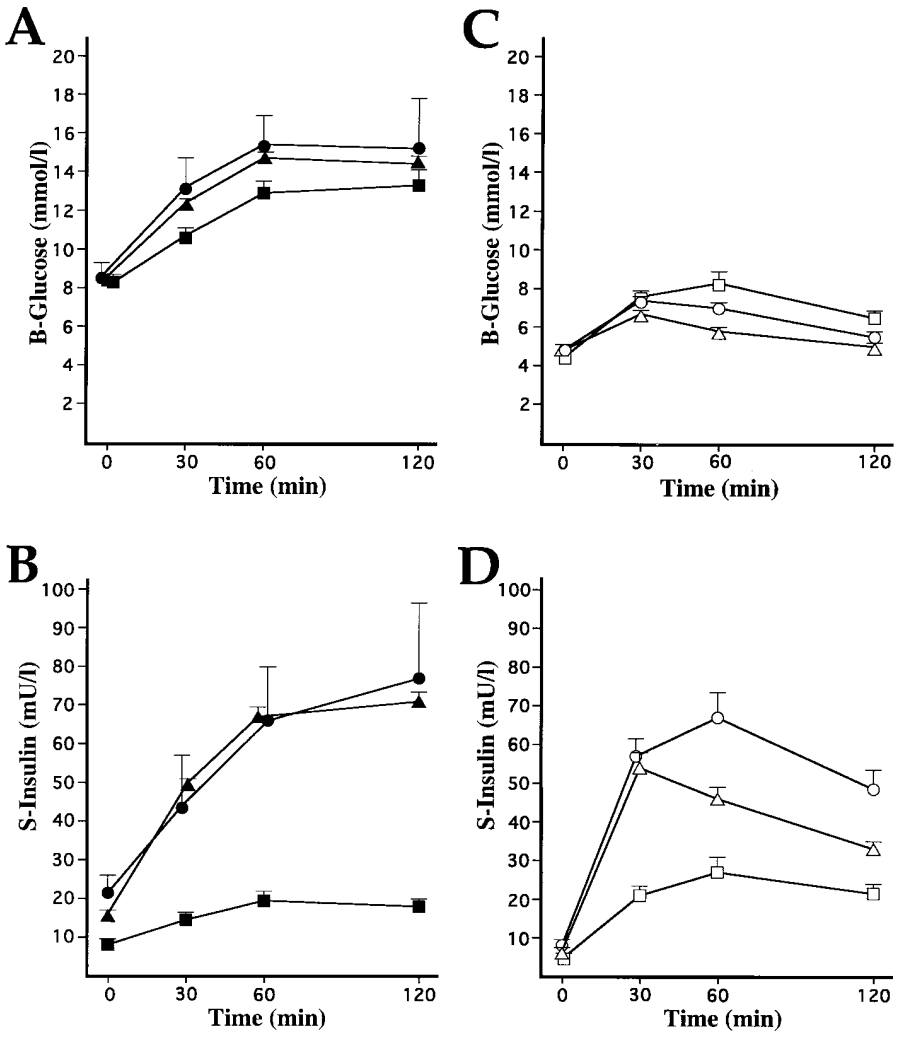

Figure 3. Measurements of blood glucose and serum insulin concentrations during OGTT in diabetic (*A* and *B*) and nondiabetic subjects (*C* and *D*) comparing MODY3 linked individuals to those not inheriting MODY3 chromosome. MODY3 patients are indicated by filled squares, NIDDM patients by filled circles, and Botnia NIDDM patients by filled triangles. MODY3 carriers are indicated by open squares, family controls are indicated by open circles, and Botnia controls are indicated by open triangles. Values are presented as mean±SEM.

The most dramatic distinction between the MODY3 patients and NIDDM patients in these families was the severe impairment of insulin secretion observed both as decreased fasting (6.2 [7.2] vs. 19.0 [20.9] mU/liter; $P < 0.001$) and incremental (1,293 [1,092] vs. 4,278 [6,056] mU/liter; $P < 0.002$) insulin concentrations during the OGTT (Fig. 3). The MODY3 patients had also significantly lower fasting (6.2 [7.2] vs. 12.6 [11.2] mU/liter; $P < 0.001$) and incremental (1,293 [1,092] vs. 3,780 [4,411] mU/liter; $P < 0.002$) insulin concentrations when compared with the Botnia NIDDM patients. MODY3 patients also had lower PI concentrations at fasting (6.9 [10.4] vs. 30.5 [41.8] pmol/liter; $P = 0.0001$) and 2 h after OGTT (23.2 [17.2] vs. 99.6 [87.1] pmol/liter; $P = 0.008$) compared with NIDDM patients.

Clinical characteristics of the MODY3 carriers. The nondiabetic MODY3 carriers ($n = 18$) were younger ($P = 0.001$) and had a lower BMI ($P = 0.001$) and fat mass ($P = 0.02$) and a greater glucose area during OGTT ($P = 0.001$) than the family controls (Table I, Fig. 3). Of note, the MODY3 carriers had significantly lower fasting (4.8 [2.0] vs. 6.4 [5.0] mU/liter; $P < 0.003$) and incremental (1,936 [1,290] vs. 3,994 [3,115] mU/liter; $P < 0.0001$) insulin concentrations compared with the family controls. The MODY3 carriers also had significantly lower fasting (4.8 [2.0] vs. 5.8 [4.4] mU/liter; $P < 0.003$) and incremental (1,936 [1290] vs. 3,612 [2600] mU/liter; $P < 0.0001$) insulin concentrations when compared with Botnia controls.

Fasting PI concentrations (4.4 [2.4] vs. 7.2 [7.0] pmol/liter) were also lower in MODY3 carriers compared with family controls ($P = 0.027$), whereas the difference in 2-h PI concentrations (23.9 [25.7] vs. 47.4 [47.3] pmol/liter) did not reach statistical significance ($P = 0.07$).

Fig. 4 shows the insulin and glucose responses during the OGTT in individuals with the MODY3 chromosome compared with data from the Botnia study (including both diabetic and nondiabetic individuals, $n = 2677$). The insulin response follows the well-known bell-shaped curve with an increase up to glucose levels of 9 mmol/liter, at which level the insulin response starts to decline. In subjects with the MODY3 chromosome, there was no increase in the insulin response to increasing glucose concentrations during the OGTT in this cross-sectional study.

To obtain a measure of insulin secretion independent of gut glucose absorption, we measured the insulin response to an IVGTT and related it to the degree of insulin sensitivity (Table III). Despite a similar degree of insulin sensitivity, there was a marked reduction in the first and the late phase of insulin secretion in MODY3 carriers (both $P = 0.0001$). Of note, this difference remained when adjusted for differences in insulin sensitivity, indicating that the low insulin response was not a function of increased insulin sensitivity. As this analysis included both diabetic and nondiabetic individuals, we also compared insulin secretion data between a subgroup of nondiabetic MODY3 carriers ($n = 6$) and Botnia controls ($n = 226$). First phase insulin secretion was clearly reduced in the MODY3 carriers compared with the Botnia controls (4.4±0.3 vs. 5.3±0.1 mU/liter × 10 min; $P = 0.004$), despite similar degree of insulin sensitivity (6.3±2.2 vs. 6.4±0.2 mg/kg min).

We further plotted insulin secretion against insulin sensitivity in those subjects who had these measurements performed during the same test. While individuals without the MODY3 chromosome showed the expected increase in insulin concentrations with increasing insulin resistance, this increase was virtually lacking in the individuals with the MODY3-chromosome (Fig. 5). Taken together the data clearly point at a defect in β cell function, which is not simply a consequence of an increased insulin sensitivity.

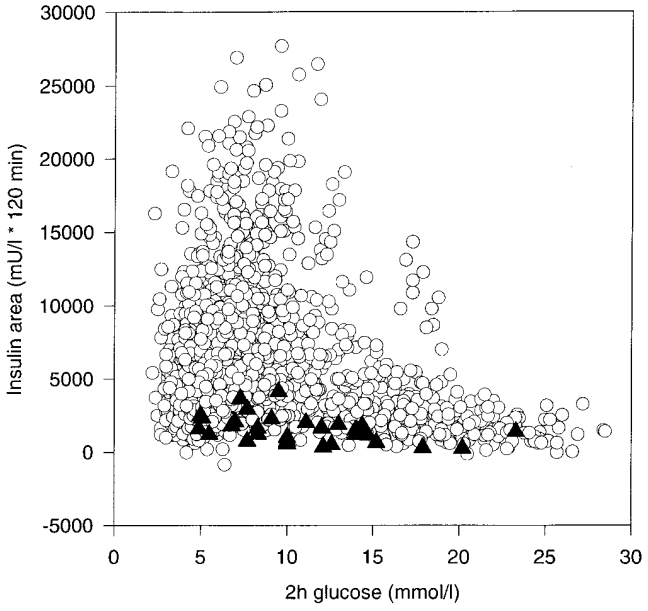

Figure 4. Incremental insulin secretion during an OGTT is plotted against the 2-h glucose concentration during the test in 30 subjects with (*closed triangles*) and in 2,677 subjects without (*open circles*) the MODY3 chromosome.

Table III. First (0–10 min) and Late (10–60 min) Phase Insulin Secretion Measured during an IVGTT and Insulin-stimulated Glucose Metabolism Measured during a Euglycemic Insulin Clamp in Subjects with (MODY3+) and without the MODY3-chromosome (MODY−) (Including Both Diabetic and Nondiabetic Subjects)

	MODY3−	MODY3+	*P* value
n	264	14	
FBG (mmol/liter)	5.8±0.1	6.4±0.7	
BMI (kg/m^2)	27.0±0.3	23.8±0.8	0.001
Age (yr)	51±1	37±3	0.001
First phase insulin secretion (mU/liter × 10 min)	5.3±0.8	3.9±0.8	0.00001 (0.0034*)
Late phase insulin secretion (mU/liter × 50 min)	7.0±0.5	6.3±0.5	0.0001 (0.0015*)
Insulin-stimulated glucose metabolism (mg/kg LBM min)	7.9±0.3	10.0±1.0	

Values are presented as mean±SEM. For testing the significance of difference between the two groups, the values were adjusted for age, BMI, and glucose tolerance with analysis of covariance. *The values for first and late phase insulin secretion were adjusted for differences in insulin sensitivity in the subgroup of individuals with values available on insulin-stimulated glucose metabolism.

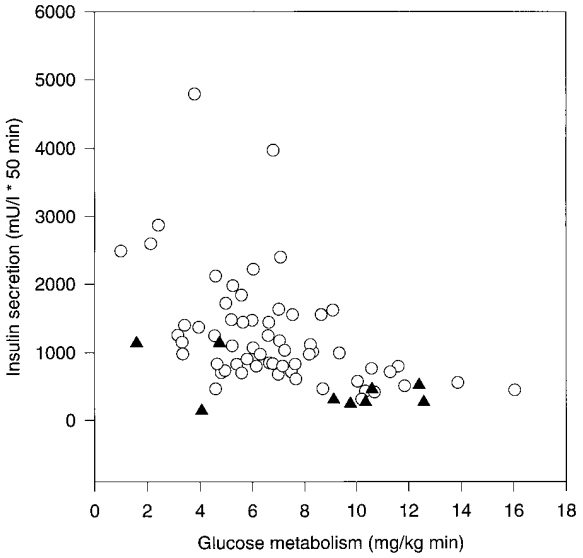

Figure 5. Insulin secretion in relation to insulin sensitivity. The insulin response during an IVGTT (10–50 min) is plotted against the rate of insulin-stimulated glucose metabolism in 9 subjects with (*closed triangles*) and in 61 subjects without (*open circles*) the MODY3 chromosome.

Discussion

The study provides strong evidence for linkage between a diabetic phenotype characterized by impaired insulin secretion and a region on chromosome 12 in four multiplex Finnish families with early-onset diabetes. Second, this diabetic phenotype is different from the common forms of NIDDM and IDDM. Third, the similarity of the phenotype with that of NIDDM2 points at the possibility that the two diseases may be caused by different alleles of the same gene. Fourth, even in this relatively homogenous Finnish population, marked heterogeneity of diabetes was observed within the same family. Fifth, screening for this form of diabetes may be justified in children at risk.

Both diabetic and nondiabetic individuals with the MODY3 gene showed impaired insulin secretion, even when adjusted for differences in age, BMI, glucose tolerance, and degree of insulin sensitivity. More than 98% of diabetic patients inheriting the MODY3-chromosome had low stimulated insulin levels. In these families, we calculated that the penetrance is 76% with respect to the phenotype of NIDDM, and 85% when phenotypes of IGT and previous history of gestational diabetes were considered.

Three of the four MODY3 families were consistent with the segregation of a single ancestral mutation. Moreover, the MODY3 phenotype segregating in all four families appeared identical and consistent with previous evidence, implicating an insulin secretory defect (15). Vaxillaire et al. reported a similar impairment in insulin secretion (measured as 2-h insulin values during OGTT) in MODY2, MODY3, and MODY patients unlinked to chromosomes 7 and 12, but did not compare their findings with insulin secretion in common NIDDM (15). Of particular interest is the finding that the MODY3 patients could not increase their insulin secretion in response to an increase in glucose. Although the data are cross-sectional, the large number of observations provide strong evidence that the MODY3 carriers do not in this respect behave like patients with early NIDDM.

The most striking finding was the fact that nondiabetic carriers of the MODY3 mutation also displayed low insulin levels (both at fasting and throughout the OGTT). Of importance, the decrease in insulin secretion must reflect a true β cell defect and cannot be explained by an increase in insulin sensitivity. The lack of GAD-antibodies in the MODY3 patients also indicates that autoimmune processes are unlikely to be involved in the pathogenesis of the β cell defect. Furthermore, the proportional decrease in PI concentrations in MODY3 patients points at a defect in some earlier step of insulin secretion rather than in the processing of PI.

Recently, Byrne et al. (25) reported normal insulin secretion rates in response to graded intravenous glucose concentrations in nondiabetic MODY3 subjects at plasma glucose levels < 8 mmol/liter. The present findings of impaired insulin responses to intravenous glucose in the MODY3 carriers with normal glucose levels do not seem to support this finding. One explanation for this difference could be heterogeneity; it is possible that different mutations in the same gene may cause β cell defects of varying severity.

There are no obvious candidate genes for an insulin secretory defect in the shared chromosomal region apart from the gene encoding for pancreatic phospholipase A2 (*PLA2*) (26, 27). Given the putative role of *PLA2* in insulin secretion, we sequenced the four published exons of the *PLA2* gene. No evidence for structural mutations was detected in the sequence of *PLA2* from two MODY3 patients (one each from families A and B; data not shown).

Another striking finding was the marked within-family heterogeneity even in this relatively homogeneous population. Therefore, it was encouraging to learn that genotypic heterogeneity was matched by phenotypic heterogeneity. Patients with MODY3 could be distinguished from patients with common NIDDM by lower BMI ($\sim$ 75% of MODY3 patients had a BMI < 27 kg/m^2), more severe impairment of their insulin secretion, less insulin resistance, lower blood pressure, and higher HDL-cholesterol concentrations. Taken together, the data indicate that the insulin resistance syndrome (syndrome X) is not part of the MODY3 phenotype.

Obviously it had been difficult to discriminate clinically between MODY3 and IDDM, as most children with MODY3 had been given a diagnosis of IDDM and treated with insulin from presentation. The presence of GAD-antibodies seems to be a good discriminator, since both children with IDDM but without the MODY3 chromosome (family A; subject IV-9, and family D; VI-19) were GAD-antibody positive. In contrast, none of the MODY3 diabetic patients had GAD-antibodies. However, one must bear in mind that of patients currently classified as IDDM at least 20% are GAD-antibody negative and 5–10% are negative for all IDDM-associated antibodies (28).

MODY3 has been estimated to account for 30% of all MODY cases in France (15). That MODY3 families have been identified subsequently in Germany, Japan, the United Kingdom, the United States (29), and now in Scandinavia suggests that MODY3 may be a fairly wide-spread form of early-onset diabetes. In fact, the prevalence of MODY3 may be substantially higher than previously thought, given the wide range in age at onset (7–56 yr) and the possible misdiagnosis of MODY3 as NIDDM or IDDM. Until the gene has been cloned, a better

estimate of the prevalence of MODY3 might be obtained by identifying the subset of diabetic patients with low insulin secretion.

We have recently mapped a gene for NIDDM (NIDDM2) also associated with an insulin secretion defect to precisely this region of chromosome 12. The similarity between phenotypes in MODY3 and NIDDM2 raises the intriguing possibility that the two diseases represent distinct alleles of the same gene which differ primarily in their age at penetrance (average age at onset 26 yr for MODY3 and 58 yr for NIDDM2). Consistent with our model of a stronger MODY3 allele, MODY3 patients display a more severe insulin secretion defect than NIDDM2 patients.

What is the risk for an unaffected carrier of the risk chromosome to develop diabetes? In women, the disease manifested frequently during pregnancy. If the MODY3 carriers are unable to increase their insulin secretion in response to a decrease in insulin sensitivity, this may create an especially severe problem during pregnancy, when women become severely insulin resistant. Our data of 10 times higher frequency of newly diagnosed diabetes in the MODY3 carriers than in the family controls (31 vs. 3%) seem to justify a recommendation of annual screening for diabetes in persons at risk. Of course, this is based upon the assumption that hyperglycemia will have a similar impact on microvascular complications in MODY3 as in NIDDM and IDDM.

In conclusion, MODY3 represents a distinct diabetic phenotype, characterized by autosomal dominant inheritance, normal weight, impaired β cell function, lack of GAD-antibodies, and absence of most features of the insulin resistance syndrome. Taken together with our earlier data on the mapping of NIDDM2, this work demonstrates the importance of phenotypic classification in studies of diabetes. It also points to a gene on chromosome 12 responsible for an insulin secretory defect as the primary event in the pathogenesis of both early (MODY3) and adult-onset NIDDM.

Note added in proof: After the acceptance of this paper, the MODY3 gene has been cloned (34). Sequencing of the hepatic nuclear factor-1α (HNF-1α) gene revealed a missense mutation (T260M) in exon 4 in family A and an insertion of nucleotide C (P291fsinsC) in exon 4 in families B, C, and D.

Acknowledgments

We thank Dr. Philippe Froguel for information on the MODY3 locus when the project was initiated, and Dr. Mark McCarthy and Pauline Sutton for assistance. We also thank members of the Botnia families for their willingness to participate in the studies, and the Botnia Research team. The GAD65 cDNA was kindly provided by Dr. Allan Karlsen and Dr. Catherine Grubin, University of Washington, Seattle, WA.

This study was supported by grants from the Sigrid Juselius Foundation, Swedish Medical Research Council (No. 10858 to L. Groop and E. Widén), Medical Faculty of Lund University (M. Lehto, T. Tuomi, and E. Widén), Malmö University Hospital (T. Tuomi), Novo Nordisk Foundation, Finnish Diabetes Research Foundation, Albert Påhlssons Foundation (M. Lehto, T. Tuomi), and Medical Society of Finland (C. Forsblom and E. Widén), and Medical Research Council of Canada (M.M. Mahtani).

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