

CLINICAL STUDY

Genetic and clinical characterisation of maturity-onset diabetes of the young in Spanish families

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Abstract

Objective: To investigate the frequencies of the major maturity-onset diabetes of the young (MODY) subtypes in a panel of Spanish families and to assess phenotypic differences in patients with the different subtypes of MODY.

Methods: Forty-eight subjects from twenty families with clinical diagnosis of MODY were studied. They underwent a standardised clinical examination and a 75-g oral glucose tolerance test (OGTT) was performed. Estimations of insulin sensitivity (%S) and insulin secretion capacity (%B) were calculated by the computer-solved homeostasis model assessment (HOMA). Mutations in the coding regions of hepatocyte nuclear factor (HNF)-4 α /MODY1, glucokinase (GCK/MODY2) and HNF-1 α /MODY3 genes were investigated by single strand conformation polymorphism and sequencing analysis.

Results: Mutations in the GCK and HNF-1 α genes were observed in 5 (25%) and 7 (35%) families respectively. Novel mutations included R385X, M238fsdelT, V226fsdelTinsAA and S418–7del11 in the GCK gene, and S121fsdelC, V133M, R159Q and V259D in the HNF-1 α gene. No MODY1 families were found. Subjects which were neither MODY2 nor MODY3 (MODY-X) had a higher fasting glucose than subjects in the other groups. Insulin secretion capacity was similar in the three groups and the insulin sensitivity was decreased in MODY-X subjects. Glucose levels were significantly higher and insulin levels significantly lower, throughout the OGTT, in MODY3 compared with MODY2 subjects.

Conclusions: Mutations in the GCK/MODY2 and HNF-1 α /MODY3 genes account for the majority of cases in a panel of Spanish MODY families, with MODY3 being the most frequent subtype. The relative frequencies and the clinical characteristics of these MODY subtypes are in agreement with data previously reported in other European populations. MODY-X patients seem to present a heterogeneous clinical profile.

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Introduction

Maturity-onset diabetes of the young (MODY) is defined as non-insulin-dependent diabetes mellitus with an early onset (usually <25 years), autosomal dominant inheritance and primary defects of insulin secretion (1). MODY is not a rare disorder and may account, in Europe, for about 2% of the subjects with type 2 diabetes mellitus (2). Furthermore, it has been shown that as many as 10% of subjects originally classified as having type 1 diabetes but without carrying high-risk human leukocyte antigen-haplotypes might indeed have MODY (3).

MODY presents genetic, metabolic and clinical heterogeneity (1), and mutations in five genes have

been shown to cause the condition. They code for the enzyme glucokinase (GCK/MODY2) (4) or for the transcription factors hepatocyte nuclear factor 4 alpha (HNF-4 α /MODY1) (5), hepatocyte nuclear factor 1 alpha (HNF-1 α /MODY3) (6), insulin promoter factor 1 (IPF1/MODY4) (7) and hepatocyte nuclear factor 1 beta (HNF-1 β /MODY5) (8). The relative prevalences of the different subtypes of MODY have been shown to vary greatly in studies of British, French and German populations (9–13). MODY2 represents from 8 to 63% of cases (the most prevalent form in France) and MODY3 from 21 to 64% of cases (the most prevalent form in England). MODY1, MODY4 and MODY5 are rare disorders, having been described only in a few families, while additional unknown

MODY locus or loci (MODY-X) represent 16 to 45% of the cases of MODY (the most prevalent form in Germany) (11–13).

No data on the relative prevalences of the different subtypes of MODY are available from countries from the south of Europe. Thus, the aim of our study was to investigate the frequencies of the major MODY subtypes in a panel of Spanish families with early onset diabetes mellitus, and to assess phenotypic differences in patients with the different subtypes of MODY.

Research design and methods

Subjects

Forty-eight diabetic members of twenty families were studied. These subjects were selected from the out-patient clinic of the Endocrinology and Diabetes Unit of the Hospital Clinic of the University of Barcelona. They presented with clinical diagnosis of MODY, including onset before 25 years of age and familial non-insulin-dependent diabetes consistent with an autosomal dominant inheritance. Sixteen of these subjects had been treated with insulin. All subjects were tested for islet autoantibodies, including islet cell autoantibodies (ICA), glutamic acid decarboxylase autoantibodies (GAD), tyrosine phosphatase autoantibodies (IA-2) and insulin autoantibodies (IAA), and none had positive results.

Methods and calculations

All subjects had a standardised clinical examination. After a 12-h overnight fast, three basal blood samples were taken within 15 min in order to measure serum glucose and insulin. Serum C-peptide was measured in insulin-treated diabetic individuals. Afterwards, a 75-g oral glucose tolerance test (OGTT) with samples at 0, 60, 90 and 120 min, was performed. For subjects younger than 12 years or with fasting serum glucose >9 mmol/l, only fasting samples were obtained. None of the subjects who underwent an OGTT was taking any drug known to affect carbohydrate metabolism, and oral hypoglycaemic agents (OHA) were withdrawn at least 48 h before the test. The protocol for the study was approved by the local medical ethics committee, and written consent was obtained from all subjects.

The area under the curve (AUC) of glucose and insulin responses during the OGTT was calculated by the trapezoidal rule. Estimations of insulin sensitivity (%S) and insulin secretion capacity (%B) were calculated from the averaged values of the three measurements of fasting serum glucose and insulin, by means of the computer-solved homeostasis model assessment (HOMA) (14).

Serum glucose and cholesterol/triglycerides were measured by the glucose oxidase and Trinder methods

(oxidase-peroxidase) respectively. Serum insulin was determined by IRMA (Medgenix Diagnostics, Fleurus, Belgium) with coefficients of variation for within and between assays of 5.2% and 6.9% respectively. No cross reaction with proinsulin was detected.

Screening for mutations in the MODY genes

DNA was isolated from peripheral leucocytes. Mutations in the coding regions of GCK and in the coding regions and the minimal promoter of HNF-1 α were identified by single-strand conformational polymorphism analysis and sequencing of bands with abnormal mobility as previously described (15, 16). Mutations in HNF-4 α were screened by direct sequencing of the minimal promoter and the 12 exons (17).

Statistical analysis

Data are presented as means \pm s.d. ANOVA was used for comparisons between groups. When this test was significant, comparisons between pairs were made using the Scheffe test. Repeated measures analysis of variance (ANOVA) was used to compare glucose and insulin response across the OGTT. Qualitative traits were analysed by Chi squared and ordered logistic regression (type of treatment). Statistics were generated with SPSS (Statistical Package for Social Sciences) version 6.1.3. for Windows, and *P* values <0.05 were considered statistically significant.

Results

Identification of mutations in MODY genes

Mutations in the glucokinase/MODY2 and HNF1 α /MODY3 genes were observed in 5 (25%) and 7 (35%) families respectively, out of the 20 families that were studied (Table 1). Each mutation was detected in all available diabetic members of the kindred and in none of the normoglycaemic relatives when tested. Mutations in HNF-4 α /MODY1 were not detected in any family.

The glucokinase/MODY2 mutations included one missense mutation (V182M), one nonsense mutation (R385X), a deletion of one nucleotide resulting in a frameshift (M238fsdelT), a deletion of one nucleotide plus an insertion of two nucleotides also resulting in a frameshift (V226fsdelTinsAA), and an 11-bp deletion in intron 9, starting at position -7 from exon 10, removing the splice acceptor site and the first four base-pairs of exon 10 (S418-7del11).

HNF-1 α /MODY3 mutations included three missense mutations (V133M, R159Q, V259D). These nucleotide changes were looked for in 25 control subjects using the same methodology. None of these changes were found in the control group. Two other mutations resulted in a frameshift; one by a deletion (S121fsdelC)

Table 1 Mutations in MODY families.

Kindred	Exon	Codon	Nucleotide change	Amino acid change	Designation
MODY2					
1	5	182	GTG-ATG	Val-Met	V182M
1	6	226	Deletion of T/insertion of AA	Frameshift	V226fsdelTinsAA
1	7	238	Deletion of T	Frameshift	M238fsdelT
1	9	358	CGA-TGA	Arg-Stop	R385X
1	10	intron 9 nt – 7/418/419	Deletion of 11 bp	Splice acceptor site	S418-7del11
MODY3					
1	2	121	Deletion of C	Frameshift	S121fsdelC
2	2	133	GTG-ATG	Val-Met	V133M
1	2	159	CGG-CAG	Arg-Gln	R159Q
1	4	259	GTC-GAC	Val-Asp	V259D
2	4	291	Insertion of C	Frameshift	P291fsinsC

and the other by an insertion (P291fsinsC) of one nucleotide (Table 1).

Clinical profile of MODY subjects

The clinical and metabolic data of MODY2, MODY3 and MODY-X subjects are shown in Table 2. Sex distribution, average age, apparent age at diagnosis, body mass index (BMI), and levels of total cholesterol and triglycerides were similar in the three groups. The probabilities for MODY3 and MODY-X subjects to be treated with OHA/insulin were 20.6 and 24.3 times higher, respectively, than for MODY2 subjects. Fasting serum glucose was higher in MODY-X subjects than in the other two groups, although a statistically significant difference was reached only for the comparison with MODY2 subjects. Fasting insulin was highly variable in MODY-X subjects (range: 36–276 nmol/l). The average value was higher than in the other two groups, but a statistically significant difference was observed only for the comparison with MODY3 subjects. The HOMA

index of insulin secretion (%B) was similar in the three groups, and the index of insulin sensitivity (%S) was decreased in MODY-X subjects as compared with the other groups. Here again, a statistically significant difference was observed only for the comparison with MODY3 subjects. Despite the small number of MODY-X individuals, we tried to identify different subcategories. In 8 out of 14 subjects, a diminution in insulin secretion capacity (%B <70%) predominated, all of them having a BMI <27 kg/m². The rest were characterised by an insulin resistance pattern (%S <64%) and a BMI >27 kg/m² in half of them. MODY2 subjects only showed macrovascular complications (2 out of 12 subjects). In contrast, more than 30% of both MODY3 and MODY-X subjects showed some evidence of microvascular complications.

Figure 1 shows glucose and insulin levels during the OGTT in MODY2 and MODY3 subjects. The OGTT was not performed in MODY-X subjects as they presented with fasting serum glucose higher than 9 mmol/l. Glucose levels were significantly higher and

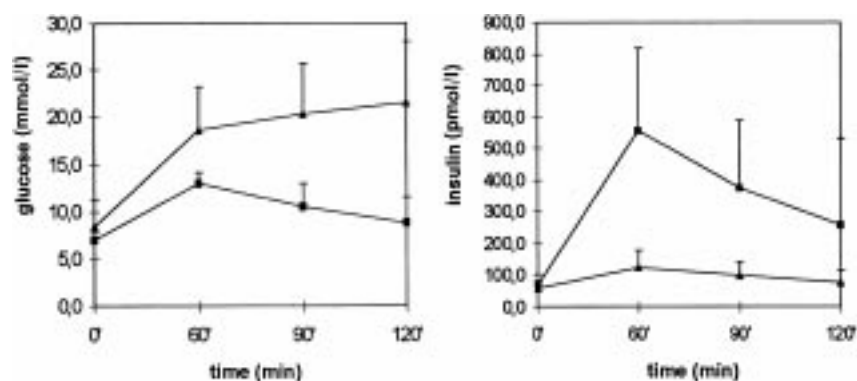


Figure 1 Serum glucose and insulin concentrations during the OGTT in MODY2 subjects (■) and MODY3 subjects (▲). Values are presented as means ± s.d.

Table 2 Clinical and metabolic profile of MODY subjects. Results are means \pm s.d.

	MODY2	MODY3	MODY-X	P
Number (men/women)	9/3	7/12	10/6	NS
Age (years)	29.8 \pm 17.2	38.8 \pm 17.3	32.1 \pm 15.2	NS
Age at diagnosis (years)	20.5 \pm 12.5	20.5 \pm 6.4	23.4 \pm 11.6	NS
BMI (kg/m ²)	24.3 \pm 5.8	23.1 \pm 3.4	25.6 \pm 4.7	NS
Total cholesterol (mmol/l)	4.1 \pm 0.8	4.9 \pm 0.8	4.7 \pm 1.1	NS
Triglycerides (mmol/l)	0.6 \pm 0.2	1.0 \pm 0.4	1.6 \pm 1.3	NS
Fasting glucose (mmol/l)	6.6 \pm 1.1*	7.9 \pm 2.4	9.1 \pm 3.0*	0.03
Fasting insulin (pmol/l)	63.0 \pm 29.4	60.0 \pm 32.4*	121.8 \pm 92.4*	0.02
AUC glucose (mmol/l.min)	1200 \pm 94	1996 \pm 514	–	0.0001
AUC insulin (pmol/l.min)	44 825 \pm 12 867	12 871 \pm 5815	–	0.0001
% B	72.5 \pm 42.5	52.8 \pm 34.4	75.5 \pm 47.8	NS
% S	80.8 \pm 28.1	98.2 \pm 30.9*	50.7 \pm 33.5*	0.005
Treatment (diet/OHA/insulin)	(10/2/0)	(5/5/9)	(2/7/7)	0.005

* Indicates the pair of values that are significantly different.

insulin levels significantly lower throughout the OGTT in MODY3 than in MODY2 subjects (repeated measures ANOVA $P < 0.0001$ for both comparisons). The AUC of glucose and insulin (time 0–120 min) express the overall responses to the oral glucose administration. The AUC of glucose was significantly higher ($P < 0.001$) and the AUC of insulin significantly lower ($P < 0.001$) in MODY3 than in MODY2 subjects (Table 2).

Discussion

We have observed relative prevalences of 25% and 35% of MODY2 and MODY3 subtypes respectively, in a panel of 20 Spanish kindreds with clinical diagnosis of MODY. The prevalence of MODY2 in this panel is closer to the prevalences reported in English (11%) (9) and German (8%) (13) kindreds, and much lower than the prevalence observed in France (63%) (12). On the other hand, the frequency of MODY3 in our sample, similar to that reported in a group of German kindreds (36%) (13), is intermediate between the frequencies observed in France (21%) (12) and in England (64%) (10, 11). No MODY1 families have been found.

These heterogeneous results may reflect distinct genetic backgrounds in these European populations and/or may be due to differences in the recruitment of families (9, 10, 13). Our panel of families was recruited through an adult clinic. It has been shown that hyperglycaemia of MODY2 often starts in childhood (18), while MODY3 is most often diagnosed after puberty or in early adult life (19).

We have not tested for mutations in the genes causing MODY4 or MODY5, as these are much less common subtypes and they are unlikely to be responsible for diabetes in a large proportion of the families from our panel. Mutations in IPF1/MODY4 were reported in only two families with MODY (7, 11), and have been excluded in panels of German (13), French (20) and Japanese (21) MODY families. As for mutations in HNF-1 β /MODY5, they have only been found

in two Japanese kindreds (8, 22) and have not been detected in other populations so far (11–13). It is noteworthy that diabetes in MODY5 patients seems to be associated with severe polykystic renal disease, which is not observed in the families that we have studied. Thus, the prevalence of MODY-X in our sample (~40%) would be closer to the values found in Germany (45%) (13), and higher than the values found in the French (16%) (12) and English (19%) (11) studies.

Four novel mutations and one previously described mutation in the glucokinase gene were found in this study. They are all likely to cause the MODY phenotype in the families. The nonsense mutation (R385X) and the two mutations resulting in a frameshift (V226fsdel-TinsAA, M238fsdelT) generate truncated proteins, and it has been shown truncated GCK lacks enzymatic activity (23). The mutation removing the splice acceptor site and the first four base-pairs of exon 10 (S418–7del11) probably alters the processing of glucokinase messenger RNA. As for the missense mutation in exon 5 (V182M), it was previously shown to co-segregate with diabetes in two French MODY families (4) and to present decreased enzymatic activity (23).

Regarding MODY3, four novel mutations and one previously described mutation in the HNF-1 α gene were found in our study. The two mutations resulting in frameshift (S121fsdelC, P291fsinsC) generate truncated proteins that probably present severely impaired transcriptional activity (24). The P291fsinsC in exon 4, which was found in two families, suggesting a possible founder effect, have previously been reported to co-segregate with diabetes in a German family (13). The V133M found in two families, the R159Q and the V259D are novel missense mutations, detected only in the diabetic members of their kindreds and in none of the normoglycaemic relatives (Fig. 2). They are likely to be mutations rather than polymorphisms. The valine at codons 133 and 259, and the arginine at codon 159 are located in conserved stretches of the HNF-1 sequence of several species (human, rat, mouse,

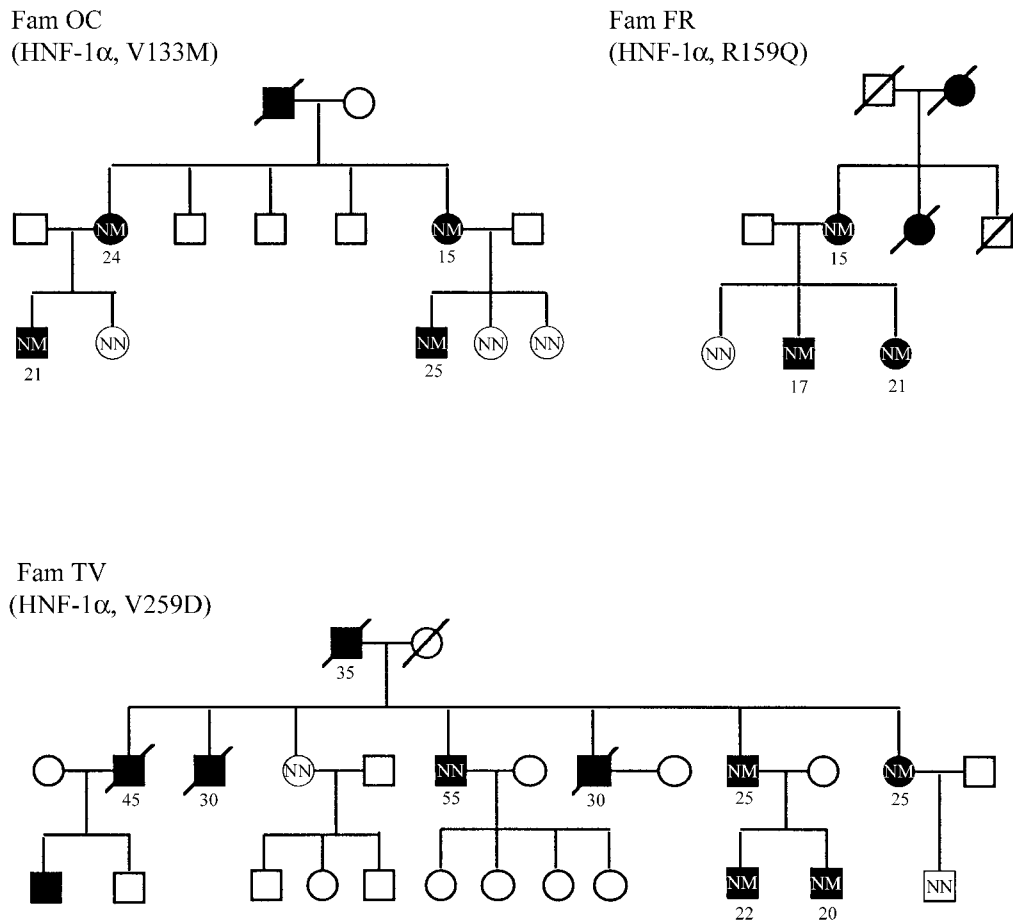


Figure 2 Pedigrees of the families with novel missense mutations in the HNF-1 α gene. Diabetic subjects are indicated with solid black symbols. Nondiabetic subjects and subjects with unknown affection status are indicated with open symbols. The genotype of family members, if known, is indicated: N, normal allele; M, mutant allele. Age at diagnosis (years) is indicated below the symbol. Squares represent males, circles represent females.

hamster, salmon), as well as in the sequences of the functionally and structurally related human and mouse HNF-1. The R159Q results in the replacement of a neutral polar amino acid (glutamine) for a basic charged amino acid (arginine), and another missense mutation in the same codon (R159W) was found to co-segregate with diabetes in an English family (10). The V259D is located in the homeobox of the DNA binding domain of HNF-1 and it results in the replacement of an acidic amino acid (aspartic acid) for a neutral and hydrophobic amino acid (valine). Both valine and methionine are neutral and hydrophobic, so the V133M does not result in a change of charge or polarity. Functional assays with *in vitro* assessment of the transactivation potential of these mutants (24) would be important to confirm their role in the pathogenesis of the diabetes.

It is noteworthy that after direct sequencing we did not detect a mutation in the coding regions, exon/intron boundaries or the promoter of HNF-1 α in one

family for which there was evidence for positive linkage to the MODY3 locus. Analysis with the D12S321 microsatellite in chromosome 12q suggested linkage with a lodscore of 3.07 (data not shown). This could be explained by the presence of another diabetes gene sharing linkage with the HNF-1 gene in the same region of chromosome 12q (25). However, we cannot exclude non-amplification of the allele containing the mutation (allelic drop out) due to a polymorphism in the PCR primers, as it has been reported for exon 2 (26). Although we have amplified exon 2 with the new set of primers that do not contain the polymorphism, we did not look for polymorphisms in the primers used to amplify the other exons and the promoter region.

The clinical characteristics of the MODY2 and MODY3 subjects are similar to those described in other populations. It has been reported that subjects with MODY2 have a mild form of hyperglycaemia (18). Insulin secretion defect in glucokinase-deficient subjects is characterised by a rightward shift in the

dose–response curve relating glucose and insulin secretion (27). On the other hand, MODY3 subjects have a severe form of diabetes characterised by severe insulin secretory defects (19, 28). During OGTT, we have observed significantly higher glucose levels and significantly lower insulin levels in MODY3 than in MODY2 subjects. Moreover, MODY3 subjects were more often treated by oral hypoglycaemic agents or insulin than MODY2 subjects. These results confirm the severe impairment of insulin secretion in response to glucose in carriers of HNF-1 α mutations. Considering chronic diabetic complications, it should be mentioned that some MODY3 subjects had developed microvascular complications while none of the MODY2 subjects did. Instead, two MODY2 subjects had suffered from macrovascular diseases.

Two different phenotypic subcategories were identified in MODY-X subjects. However, this piece of information should be considered with caution because of the small number of subjects. Around 60% of them displayed predominantly a decreased insulin secretion capacity, while in the rest some features of an insulin-resistance pattern could be identified. On average, MODY-X subjects had a more severe hyperglycaemia than MODY2 and MODY3 subjects, with higher levels of fasting insulin. These results contrast with those observed in French MODY families, where it was found that MODY-X subjects present, on average, a milder form of diabetes than MODY3 subjects (12, 19). On the other hand, a recent study of American subjects with early-onset autosomal-dominant type 2 diabetes not related to known MODY genes shows that these patients frequently present a decrease in insulin sensitivity (29).

In conclusion, mutations in the glucokinase and HNF-1 α genes account for the majority of cases of MODY in a panel of Spanish families, with MODY3 being the most frequent subtype. The relative frequencies and the clinical characteristics of these MODY subtypes are in agreement with data previously reported in other European countries. Patients with MODY-X, caused by mutations in still unknown locus or loci, seem to present heterogeneous clinical profiles. Identification of the remaining MODY genes will permit the sorting out of the clinical and metabolic characteristics of more homogeneous sub-groups of patients.

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