0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(6):2532–2539

Nine Novel Mutations in Maturity-Onset Diabetes of the Young (MODY) Candidate Genes in 22 Spanish Families

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The aims of this study were to estimate the prevalence of major maturity-onset diabetes of the young (MODY) subtypes in Spanish MODY families and to analyze genotype-phenotype correlations. Twenty-two unrelated pediatric MODY patients and 97 relatives were screened for mutations in the coding region of the glucokinase (GCK), hepatic nuclear factor-HNF-1α and HNF4α genes using PCR-single strand conformation polymorphism and/or direct sequencing. In families carrying GCK mutations, the influence of genetic defects on fetal growth was investigated by comparing the birth weights of 32 offspring discordant for the mutations. Mutations in MODY genes were identified in 64% of the families. GCK/MODY2 mutations were the most frequently found, in 41%: seven novel (R369P, S411F, M298K, C252Y, Y108C, A188E, and S383L) and 2 already described mutations. Four pedigrees (18%) harbored mutations in the HNF-1α/MODY3 gene, including a previously unreported change (R271G). One family (4%) carried a novel mutation in the HNF-4α gene (IVS5-2delA), representing the first report of a MODY1 pedigree in the Spanish population.

The age at diagnosis was prepubertal in MODY2 index patients and pubertal in MODY3 patients. Overt diabetes was rare in MODY2 and was invariably present in MODY3 index patients. Chronic complications of diabetes were absent in the MODY2 population and were present in more than 40% of all relatives of MODY3. Birth weight was lower in the presence of a GCK fetal mutation when the mutation was of paternal origin. The MODY1 patient was diagnosed at 15 yr of age. She developed intermittent microalbuminuria despite good metabolic control, and severe late-onset complications were common within her family.

Mutations in the GCK/MODY2 gene are the most common cause of MODY in our population as recruited from pediatric and adolescent index patients. The inheritance of GCK defects by the fetus results in a reduction of birth weight. Clinical expression of MODY3 and MODY1 mutations, the second and third groups of defects found, was more severe, including the frequent development of chronic complications. (*J Clin Endocrinol Metab* 87: 2532–2539, 2002)

MATURITY-ONSET diabetes of the young (MODY) is a genetically, metabolically, and clinically heterogeneous type of noninsulin-dependent diabetes mellitus. It is characterized by early onset (usually before 25 yr of age and often in adolescence or childhood), autosomal dominant inheritance and a primary defect in glucose-stimulated insulin secretion (1). Molecular defects in six different genes have been currently identified in MODY patients (2). All of these genes encode proteins involved in the glucose homeostasis of the pancreatic β-cell. The glucokinase gene (GCK/MODY2) encodes an enzyme of the glycolytic pathway, which can modulate insulin secretion in response to glycemic variations (3–5). The other five genes encode nuclear proteins that control the appropriate expression of β-cell genes the hepatic nuclear factor-4α (HNF-4α/MODY1), HNF-1α (MODY3), the insulin promoter factor-1 (MODY4), the HNF-1β (MODY5), and the NEUROD1/β2 factor (MODY6) (6–10). Heterozygous mutations in these genes appear to result in different clinical presentations. The recent identification of these MODY genes currently allows investigation of the specific defects present in each family and definition of the respective genotype-phenotype correlations (11).

Clinical heterogeneity is well established among MODY patients. Those with mutations in the GCK gene (MODY2) present with a mild form of diabetes often diagnosed in the early years of life. Although GCK mutations can hinder fetal growth and weight gain by the impairment of fetal insulin secretion (12), MODY2 patients usually maintain good glycemic control without the need for insulin and with rare development of vascular complications (13). In contrast, patients carrying mutations in the HNF-4α and HNF-1α genes (MODY1 and MODY3, respectively) generally exhibit more severe fasting hyperglycemia, a high percentage of insulin requirement, and a frequent occurrence of late-onset complications of diabetes (14, 15). Diabetes can also present in association with distinct clinical features, as the development of renal polycystic disease and malformations in the genital tract in the case of MODY5 (9, 16, 17) or an older age at diagnosis for MODY4 patients (8).

Different studies suggest that the prevalence of specific mutations of MODY genes differs considerably among various ethnic groups (18). More than 130 different GCK/MODY2 mutations have been currently identified (11). In France, GCK mutations are the most common cause of MODY, with more than 60% of studied pedigrees carrying mutations in this gene (18–20). GCK defects are also the first cause of MODY among Italian children (21). However, recent studies in the United Kingdom (22) and Germany (23) found GCK mutations in only 11% and 8% of the series, respectively. More than 120 different HNF-1α/MODY3 mutations

Abbreviations: GAD, Glutamic acid decarboxylase autoantibodies; GCK, glucokinase; HNF-4α, hepatic nuclear factor-4α; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test.

have been currently described (11, 24). In contrast with the French survey, mutations in HNF-1α/MODY3 were found to be the most common defects to cause MODY in other series (25). The only available study conducted among the Spanish population found HNF-1α/MODY3 mutations in 35% of the families analyzed, representing the most frequent subtype of MODY, and GCK/MODY2 mutations in 25% of pedigrees (26).

MODY1 is a rare cause of diabetes (6), initially reported in a large pedigree (1) and more recently in few other reports (27–30). In a study conducted in the U.S. of 53 Caucasian families, HNF-4α mutations only accounted for an overall prevalence of 2–4% (10). MODY1 has not been described in families of Spanish descent. The MODY5 subtype is associated with renal malformations and renal dysfunction in families carrying HNF-1β mutations. Reports on defects causing MODY4, MODY5, and MODY6 are still very rare (8). Recently, their respective prevalence has been reported to be 0%, 1%, and 0% in a cohort of 90 United Kingdom (25) Caucasian families. The relative distribution of rare subtypes of MODY in different ethnic backgrounds has yet to be determined.

Although recent studies suggest that MODY is not uncommon among the general diabetic population (31), little information is available on the prevalence of MODY and MODY subtypes among children and adolescents. Recently, we reported that 5% of diabetic patients controlled at our Pediatric Diabetes Unit presented diagnostic features of MODY (32).

The aim of the present study was to assess the nature and frequency of mutations in the genes responsible for most common types of MODY (MODY1, -2, and -3) in 22 unrelated Spanish families recruited from pediatric index population. After the molecular classification of pedigrees, clinical and metabolic phenotypes were analyzed within each ethiological group, including the influence of maternal/fetal genotypes on fetal growth in the case of GCK mutations.

Experimental Subjects

Twenty-two unrelated pediatric and adolescent patients with clinical features of MODY were included in the study. They were recruited from the out-patient clinic of the Pediatric Diabetes Unit at the Ramon y Cajal Hospital (Madrid, Spain), covering a general population more than 500,000 people, of whom more than 85,000 were under 20 yr of age. At the time of investigation, the ages of the patients ranged from 2 months to 20 yr (mean, 11.6 $\pm$ 4.7). Clinical history and metabolic and genetic studies were performed in patients as well as in 97 relatives available from the 22 families. All pedigrees contained 1 or more individuals known to have diabetes mellitus, impaired fasting glucose (IFG), or impaired glucose tolerance (IGT) in at least 3 consecutive generations. Families were all unrelated and were from Caucasian Spanish descent. Written consent was obtained from all participants or from responsible family members.

Materials and Methods

Metabolic studies

Fasting plasma glucose and hemoglobin A_{1c} were determined in all probands and pediatric relatives, and in the absence of overt diabetes, two oral glucose tolerance tests (OGTT) were performed. Two hours after the oral ingestion of 1.75 g glucose/kg (maximum, 75 g), glucose and insulin levels were measured. According to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (33), patients were classified into three groups: diabetes, IGT, or IFG.

Probands were tested for insulin autoantibodies, tyrosine phosphatase autoantibodies (IA-2), glutamic acid decarboxylase autoantibodies (GAD), and islet cell autoantibodies.

Diagnosis of chronic diabetes complications was based on standard studies (serum lipid profile, blood pressure, microalbuminuria, and eye fundoscopy). In adult relatives we studied fasting glycemia and glycemia 2 h after breakfast. The existence of chronic complications in affected relatives was assessed from medical files.

Studies of birth weight

In pedigrees affected by GCK mutations, mothers were asked for details of their pregnancies and birth weights of their offspring. Children within these families were divided into four groups according to the presence of the mutation: 1) only in the mother (group A), 2) in both the mother and the child (group B), 3) in neither the mother nor the child (group C), and 4) only in the child (group D). Mean birth weights among the groups were compared. Within each MODY2 family, birth weights of sibling pairs who were discordant for the presence of the mutation were also compared. Data from children born after complicated pregnancies were excluded.

Genetic studies

For mutation screening, genomic DNA was isolated from peripheral blood lymphocytes of patients and relatives. The genetic strategy was the screening of MODY genes in probands, followed by the investigation of relatives by sequencing when a mutation was identified. The complete coding sequence (along with flanking intronic boundaries) of the GCK and HNF-1α genes was screened in probands by fluorescent-based PCR-single strand conformation polymorphism and direct sequencing. PCR products corresponding to abnormal electrophoretic patterns were directly sequenced to characterize nucleotide variants on an automated ABI 377 sequencer (Perkin-Elmer Corp., Foster City, CA) (34). Probands negative for GCK and HNF-1α genes after the PCR-single strand conformation polymorphism screening were confirmed by sequencing. The HNF-4α gene was screened by direct sequencing (6). The presence of mutations in relatives of MODY patients was investigated by direct sequencing of the affected exon.

All laboratory analyses were performed with commercially available standardized methods. Fasting plasma glucose was measured using the glucose oxidase method (glucose autoanalyzer, Beckman Coulter, Inc., Brea, CA). Hemoglobin A_{1c} was determined using HPLC (Menarini HA81-10; normal, 4.04 $\pm$ 0.35%). Insulinemia was determined using an RIA procedure (Sorin, Biomedica Saluggia, Italy; normal, 58–187 pmol/liter). Insulin autoantibodies were determined using a competitive fluid phase RIA that uses ^{125}I-labeled, recombinant human insulin (Amersham Pharmacia Biotech, Arlington Heights, IL) as antigen and polyethylene glycol for precipitation of immune complexes (normal, $<$40 nU/ml). IA-2 and GAD were detected using standard radiobinding assays with ^{35}S-labeled recombinant human GAD65 and IA2 antigens obtained by *in vitro* translation using cDNA clones provided by Dr. A Lernmark (University of Washington, Seattle, WA) and Dr. E. Bonifacio (Istituto Scientifico San Raffaele, University of Milan, Milan, Italy), respectively (normal values: I-A2, $<$0.02; GAD, $<$0.02).

Statistical analysis

Statistical analysis was performed using the R-Sigma statistical package. Birth weight data were compared in pairs among the four groups established based upon maternal and child GCK mutation status, using two-way ANOVA. Results are expressed as the mean $\pm$ SD. Significance was considered at $P \leq 0.05$.

Results

Identification of mutations

In this study a total of 14 mutations have been identified in 3 MODY genes in 22 families of Spanish descent, repre-

senting 64% of our population. Nine of them have not been previously described, and all of them cosegregate with the clinical phenotype of MODY within the pedigrees. Detailed characteristics of these mutations are shown in Table 1.

GCK defects. Mutations in the GCK gene were detected in 41% of the families studied. Each family carried a different defect. Mutations consisted of eight missense mutations that resulted in single amino acid substitutions (Y108C, C252Y, M298K, R369P, S411F, A188T, A188E, and S383L) and one nucleotide deletion resulting in a frame-shift deletion (M238fsdelT) that leads to a stop codon. Seven of these mutations have not been described previously (Table 1). None of these nucleotide variants was present in 84 DNA controls screened by sequencing. Within the pedigrees, mutations were present in all available subjects with hyperglycemia and were absent in normoglycemic relatives.

HNF-1α defects. Eighteen percent of the families were identified with mutations in the HNF-1α/MODY3 gene. Changes included three amino acid substitutions (R200W, R159W, and R271G) and one nucleotide deletion resulting in a frameshift (P291fsinsC). The novel missense mutation R271G was absent from the control group and cosegregated with the diabetic phenotype in the family.

HNF4α mutations. One mutation in the HNF4-α/MODY1 gene was detected (4%). The defect (IVS5 -2delA) consists of a deletion located at the canonical acceptor splice site of exon 6. It represents a novel mutation and the first HNF-4α defect described in a family of Spanish descent. This mutation was present in the index patient and two affected relatives and was absent in one unaffected subject (Fig. 1).

MODYX. In 36.5% of the families the minimal promoter and coding region of the three studied MODY genes were screened, but no mutation was found.

Clinical and metabolic features of MODY patients

Based on the results of mutation screening, clinical and metabolic profiles of pediatric patients belonging to each molecular category of MODY were analyzed and compared. Selected characteristics are summarized in Table 2.

TABLE 1. Mutations in major MODY genes in 22 Spanish families

Gene	Nucleotide change	Designation
GCK/MODY 2		
1	c.323A>G	Y108C
2	c.562G>A	A188T[a]
3	c.563C>A+564T>A	A188E
4	c.713delT	M238fsdelT[a]
5	c.755G>A	C252Y
6	c.893T>A	M298K
7	c.1106G>C	R369P
8	c.1148C>T	S383L
9	c.1232C>T	S411F
HNF 1α/MODY 3		
1	c.475C>T	R159W[a]
2	c.598C>T	R200W[a]
3	c.811C>G	R271G
4	c.872 873insC	P291fsinsC[a]
HNF 4α/MODY 1		
1	c.842-2delA	IVS5-2delA

[a] Previously reported (14, 23, 34, 38).

Pediatric patients with MODY2

The age of investigation in MODY2 pediatric patients was prepubertal (7.9 ± 3.8 yr). Their mean fasting glucose level was 6.6 ± 0.51 mmol/liter. One patient presented with IFG, 2 had IGT, 10 had IGT and IFG, and only 1 patient (7%; carrying the R369P mutation) presented overt diabetes. This diabetic patient is well controlled with oral hypoglycemic agents, and the rest are controled with diet and physical exercise. Although hyperglycemia developed early in life, the diagnosis of most probands was fortuitous.

Penetrance of the clinical phenotype was high, and the level of hyperglycemia increased significantly with age ($P < 0.05$; Fig. 1). Except for the family carrying the R369P mutation, in which several members had diabetes requiring oral hypoglycemic agents (Fig. 2), clinical and biological expressions of MODY2 relatives were homogeneously mild (within and between families) and were controlled with diet. No evidence of diabetes complications was found in affected members of these families.

Birth weight variation in the presence of maternal and fetal GCK gene mutations. In the 32 offspring with 1 affected parent, birth weight was lower in the presence of a fetal mutation when the mutation was of paternal origin (group D; Fig. 3). When both the fetus and the mother had a GCK mutation, the birth weight was 3551 ± 306 g. If the mother did not have a GCK mutation but the fetus did (group D), birth weight was 2706 ± 316 g. The difference between these 2 groups was significant ($P < 0.001$). A less significant difference in birth weight was observed when both mother and fetus were discordant (groups A and D) for their mutation status ($P < 0.01$). In 14 sibling pairs discordant for GCK mutation, the affected sibling had a significantly lower mean birth weight (mean, 525 g; $P < 0.001$).

Pediatric patients with MODY3

The mean age at diagnosis of the five pediatric patients with the MODY3 mutation was 14.4 ± 2.7 yr, and all of them had started puberty. The mean fasting plasma glucose and insulin levels were 8.09 ± 1.91 mmol/liter and 60.9 ± 24.6 pmol/liter. One patient is controlled with diet and physical exercise, two patients are controlled with hypoglycemic agents (one was too sensitive to sulfonylureas, which were replaced with repaglynide), and two are receiving insulin therapy. The overall prevalence of late complications of diabetes (nephropathy and retinopathy) in their families is high (41%). Three relatives from three different families who had inherited the mutation showed normal glucose metabolism, compatible with the described variable penetrance for MODY3 parallel to age. A detailed comparison between the characteristics of MODY2 and MODY3 pediatric patients is shown in Table 2.

Patients with MODY1

The only proband with an HNF-4α mutation was diagnosed at 15 yr of age with fasting glycemia of 6.9 and 13.5

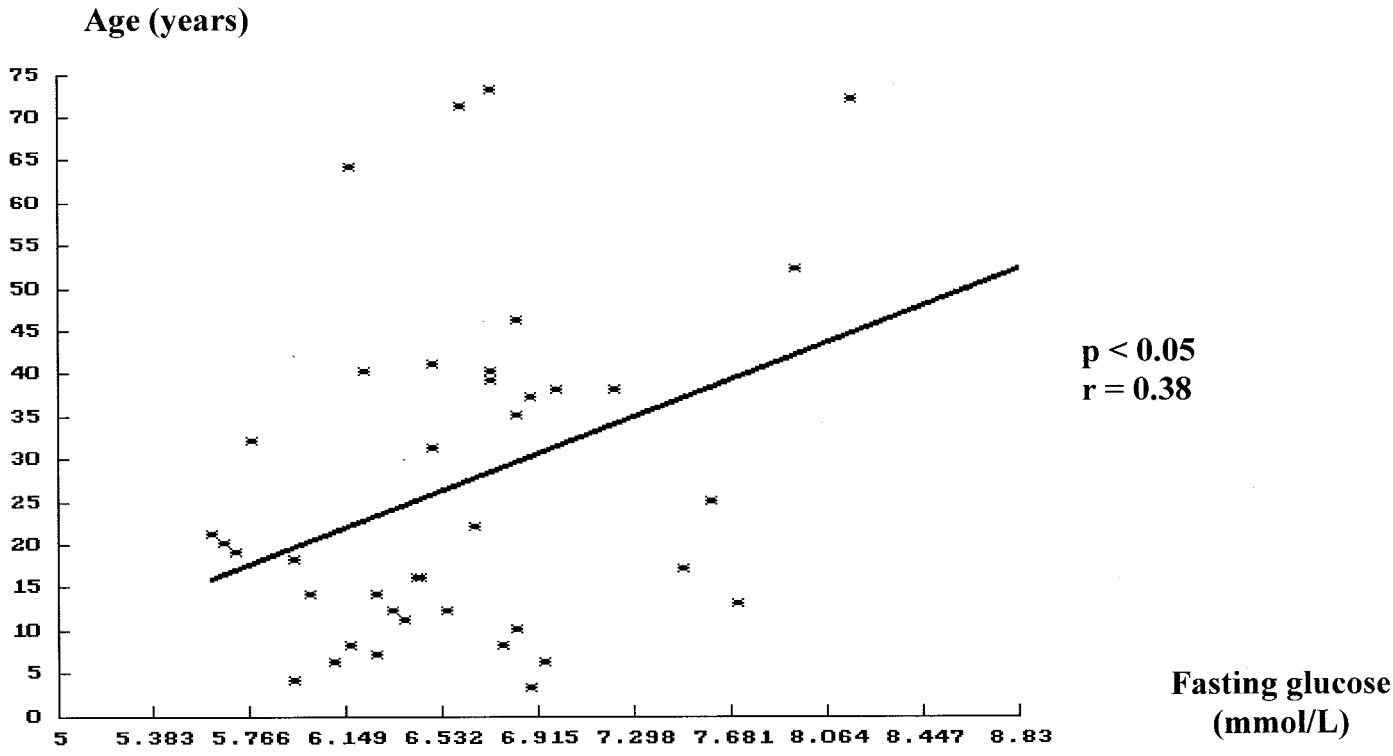

FIG. 1. Fasting glucose in family members with glucokinase mutations.

mmol/liter at 2 h in an OGTT. She was overweight (body mass index, 24.2 kg/m^2). Controlled with diet and exercise, she maintained abnormal glucose metabolism (OGTT fasting glucose, 7.5 and 9.9 mg/dl at 2 h). She presented macroalbuminuria four times during the first year after diagnosis. Proteinuria not related to diabetes was studied and excluded. In the following years she presented microalbuminuria (defined by two of three urine determinations >20 μg/min) on three different occasions. Between these periods normoalbuminuria was found. Based on this, we believe that this patient presents intermittent microalbuminuria.

One of her two normoglycemic siblings underwent genetic studies, which were negative. Her father was diagnosed with diabetes at the age of 31 yr and was treated with oral hypoglycemic agents until the age of 46 yr, when insulin treatment was started. At 25 yr follow-up, severe diabetic complications were detected: proliferative retinopathy, nephropathy, and peripheral neuropathy.

Two paternal uncles of the index patient were also diabetic with chronic diabetes complications (nephropathy); one of them died from renal failure. The paternal grandfather and several other relatives were also affected (Fig. 4).

Patients MODY-X

The mean age at diagnosis of probands with MODY-X was 14.6 ± 5.3 yr; half of them were prepubertal. The mean fasting plasma glucose and mean insulin levels were 6.17 ± 0.62 mmol/liter and 486.8 ± 939.5 pmol/liter, respectively. All patients are controlled with diet and exercise. None of the relatives had late complications of diabetes.

Discussion

MODY is caused by mutations in a reduced number of β-cell genes. Mutations in the GCK/MODY2 gene are the most common cause of MODY found in our study. We identified nine different GCK mutations. Seven of them correspond to mutations not previously described. The prevalence of MODY2 in our study (41%) is lower than that in France (63%) (19), but higher than that reported by Hattersley *et al.* (12.5%) in 40 United Kingdom MODY pedigrees (18) or by Costa *et al.* (26) (25%) in their study of 20 Spanish families recruited through an adult diabetes clinic. These contrasting results among countries and within Spain might reflect the different ways of recruitment of MODY families: from adult index patients in the studies by Hattersley *et al.* (18) and Costa *et al.* (26), mainly pediatric patients in the French study (19), and exclusively pediatric probands in our study. The prevalence of MODY types with late clinical expression might be overrepresented in series with adult recruitment and underrepresented in series with pediatric and adolescent recruitment.

Our frequency of MODY3 (18%) is similar to that reported in France (21%) (35). We found one family with MODY1; this was the first MODY1 family reported in Spain. The prevalence of MODY-X (families with MODY in which no mutations were found) was 36% in our population, comparable with those reported previously in Spain (40%) (26) and in Germany (45%) (23). The molecular background of MODY-X families remains to be determined. Other MODY candidate genes are probably not involved in the pathogenesis, as HNF-1β/MODY5 defects are associated with renal cystic

TABLE 2. Clinical and metabolic characteristics in pediatric patients with MODY1, MODY2, MODY3, and MODY-X

	MODY 1	MODY 2	MODY 3	MODY-X	ANOVA (*P*)
No. (male/female)	1 (0/1)	14 (10/4)	5 (2/3)	8 (5/3)	
Age of investigation (yr)	15	7.9 ± 3.8	14.4 ± 2.7	14.6 ± 5.3	<0.01
Duration of abnormal glucose metabolism (yr)	9	6 ± 4.6	4.0 ± 4.8	3.6 ± 3.1	
BMI (SD)	1.52	0.46 ± 1.05	−0.78 ± 0.12	1.6 ± 1.6	<0.01
Fasting glycemia/2 h (mmol/liter)	6.9/13.5	6.6 ± 0.51/9.04 ± 1.35	8.09 ± 1.91/15.95 ± 2.43	6.17 ± 0.62/8.47 ± 3.06	<0.01/<0.001
Fasting insulin/2 h (pmol/liter)	29.16/113.2	64.4 ± 34.2/227.1 ± 124.7	60.9 ± 24.6/208.7 ± 153.7	486.8 ± 939.5/402.1 ± 213.9	
HbA_1c (%) (normal, 3.5–4.5%)	4.1	5.1 ± 0.4	6.7 ± 1.5	5.2 ± 1.4	<0.05
Prevalence of diabetes (%)	100	7.14	100	28.6	
Chronic complications (%; dn, dr, dneu)	0	0	20 (dn)	0	
Treatment (diet/OHA/insulin)	1/0/0	13/1/0	1/2/2	7/0/1	

OHA, Oral hypoglycemic agents; dn, diabetic nephropathy; dr, diabetic retinopathy; dneu, diabetic neuropathy.
By Newman-Keuls test: Age MODY2/MODY3, $P < 0.01$; MODY2/MODYX, $P < 0.01$.
BMI MODY2/MODYX, $P < 0.05$; MODY3/MODYX, $P < 0.01$.
Fasting glycemia and post-OGTT MODY2/MODY3, $P < 0.01$; MODY3/MODYX, $P < 0.01$.
HbA_1c (%) MODY2/MODY3, $P < 0.05$; MODY3/MODYX, $P < 0.05$.

disease and malformations in the genital tract, a phenotype that was absent in our cohort of MODY families. Insulin promoter factor-1 or NeuroD1 defects are rarely mutated in studies performed in MODY families of Caucasian origin (23, 25).

With respect to the MODY2 phenotype, our findings of birth weight reduction in children with GCK mutations are in agreement with previous data reported by Hattersley (12) and Velho (36). In families with GCK mutations, maternal environment and fetal genotypes can alter growth *in utero* by influencing fetal insulin secretion. Maternal hyperglycemia can compensate for the effect of fetal mutations on birth weights. As these glucose-sensing defects are constitutively present in the β-cell of the affected fetuses, hyperglycemia might be present from birth.

Despite the wide heterogeneity of GCK missense mutations identified in our study, clinical expression of MODY2 was homogeneously mild, with the exception of the R369P mutation. In agreement with previous observations (19, 37), subjects with a GCK deficiency frequently have IGT rather than overt diabetes. In our study most of MODY2 pediatric patients maintained good glycemic control without the need for insulin or oral agents. The patient with the novel R369P mutation, diagnosed at the age of 8 yr, is the only MODY2 pediatric patient currently controlled with oral hypoglycemic agents. He temporarily needed insulin treatment for a surgical procedure. The R369P mutation replaces an arginine, a basic and charged amino acid that is highly conserved in several species, with a proline, a neutral and nonpolar amino acid that may reduce the stability of the glucokinase by affecting its secondary structure. Functional studies of the effect of this GCK mutation are in progress.

The effects of GCK mutations on enzyme activity and kinetics are difficult to predict base on their locations or the nature of amino acid changes (20, 38, 39). For example, a recent functional study (Cuesta A., *et al.*, unpublished) performed on the M298K mutation identified in one of our families revealed, as a novel pathogenetic mechanism, a reduction of ATP binding of the enzyme that is necessary for glucose phosphorylation. We also identified a frame-shift mutation (M238fsdelT) that was previously reported in a Spanish family and that may result from a founder effect (40).

In our cohort of index patients, MODY3 invariably presented with overt diabetes after the start of puberty. Two patients need insulin, two are controlled with oral agents, and one is controlled with diet and exercise. The incidence of chronic complications in affected relatives is high, except for the family with the P291fsinsC mutation.

The more severe phenotype of MODY3 patients compared with MODY2 reflects the profound effect of HNF-1α mutations on β-cell insulin secretion potential (37). Among the HNF-1α mutations, we identified the P291fsinsC as the most commonly described mutation in MODY3. This mutation is located in the polyC tract of exon 4 and induces a severely impaired transcriptional activity in the mutant protein. It is known to have a dominant negative effect *in vitro* by retaining its DNA-binding potential and forming nonfunctional dimmers with the wild-type HNF-1α (24, 40, 41). The three other missense mutations identified, one novel (R271G) and two previously described (R159W and R200W), are located

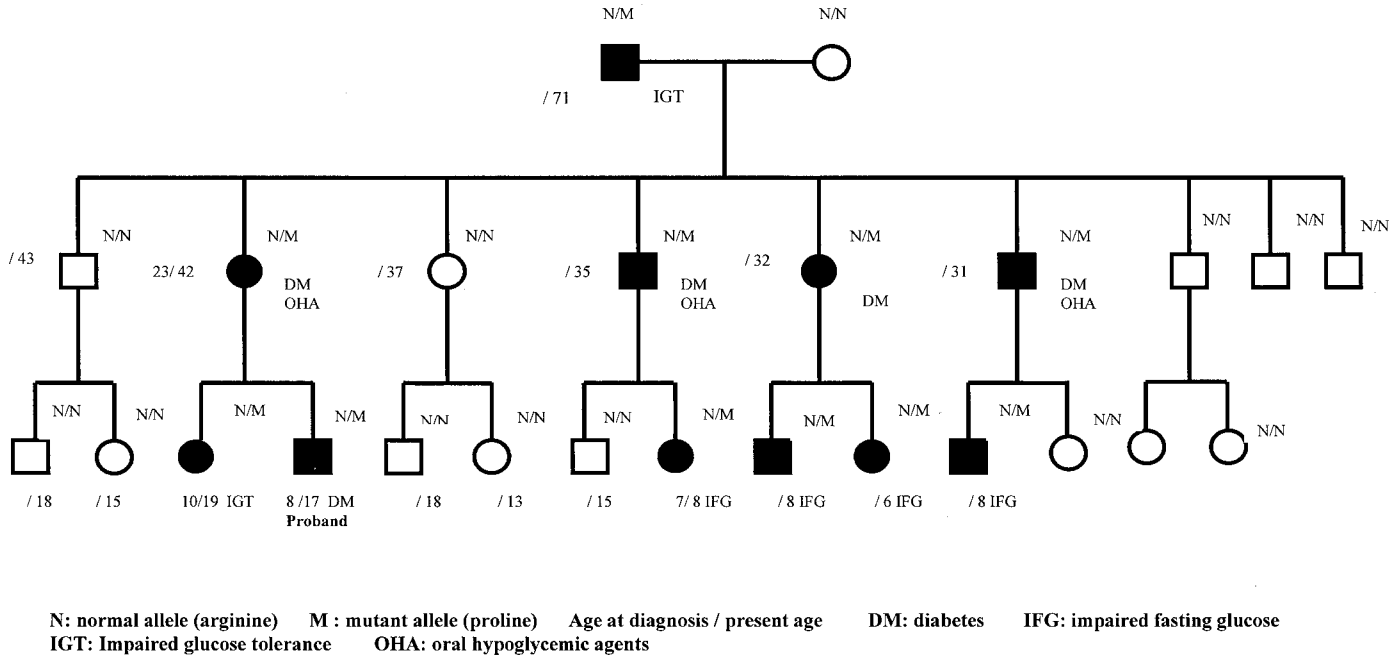

FIG. 2. Pedigree of the family with R369P GCK mutation.

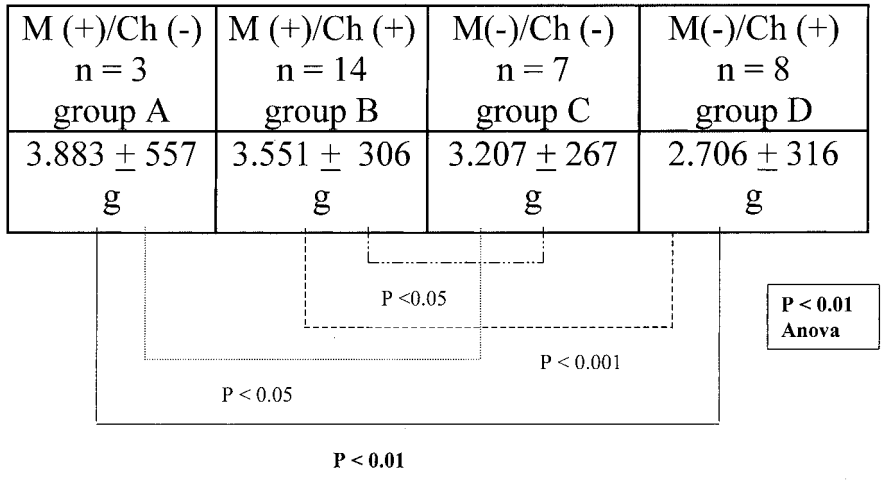

FIG. 3. Influence of maternal (M+) and child (Ch+) glucokinase mutations on birth weight.

in a CG repeat, and two of them are compatible with the deamination of methylcytosine (CG→TG) reported in a high proportion of HNF-1α point mutations. The affected residues are conserved among sequences of different species and are located in the DNA-binding domain of the protein, suggesting their important functional roles.

The only MODY1 pedigree in our series has a severe form of diabetes with a high incidence of chronic complications. Clinical expression was more delayed in life. The mutation identified is a splicing defect (IVS5–2delA) located at a conserved position of the acceptor splice site. As predicted according to the Splice Site Prediction by Neural Network, this splicing mutation leads to a factor 2 reduction of the acceptor splice site score compared with the wild-type sequence. It also induces the activation of a cryptic activator splice site 13 nucleotides downstream, leading to a peptide shortened by 12 amino acids. Further functional studies are needed to precisely define the effect of the IVS5–2deA mutant protein on HNF-4α function (42).

The MODY-X subjects had a similar age of diagnosis, significantly higher body mass index, and significantly less severe glycemia alteration than MODY3 patients without significant differences in insulinemia.

In conclusion, mutations in the GCK gene are the most common cause of MODY in our Spanish population recruited from pediatric and adolescent index patients. The

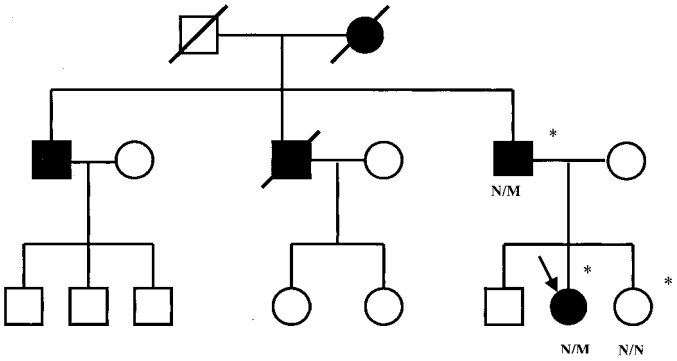

FIG. 4. Pedigree of the family with MODY1.

inheritance of GCK defects by the fetus results in a significant reduction of birth weight. The clinical expression of MODY3 and MODY1, the second and third groups of defects found, was more severe and was frequently associated with the development of chronic complications of diabetes.

Acknowledgments

We are indebted to Dr. L. Castaño for the determination of insulin, IA-2, and GAD antibodies, and to V. Carrasco, M. A. Alvarez, and R. Contreras for their technical assistance.

Received October 4, 2001. Accepted February 7, 2002.

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