HNF1α Mutations Are Present in Half of Clinically Defined MODY Patients in South-Brazilian Individuals

clinical case report

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ABSTRACT

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance, early age of onset, and pancreatic beta cell dysfunction. Heterozygous mutations in at least seven genes can cause MODY. In the present study we investigated the relative prevalence of *GCK* (glucokinase) and *HNF1α* (hepatocyte nuclear factor 1α) mutations, the more frequent causes of MODY, in 13 South-Brazilian families with multiple cases of diabetes consistent with MODY. Heterozygous variants in *GCK* and *HNF1α* genes were observed respectively in one (7.7%), and six (46.2%) families. The six *HNF1α* variants are likely to cause diabetes in the families where they were observed. However, we could not ascertain whether the GCK Gly117Ser variant found in one family is a causal mutation. In conclusion, we have confirmed in a South-Brazilian population that *HNF1α* mutations are a common cause of monogenic diabetes in adults selected with strict clinical diagnostic criteria. **(Arq Bras Endocrinol Metab 2008; 52/8: 1326-1331)**

Keywords: Maturity-onset diabetes of the young; Monogenic diabetes; Transcription factor; Glucokinase; Hepatocyte Nuclear Factor 1α

RESUMO

Mutações HNF1α Estão Presentes na Metade dos Pacientes da Região Sul do Brasil Clinicamente Diagnosticados com MODY.

O *maturity-onset diabetes of the young* (MODY) é uma forma monogênica de diabetes melito caracterizada por herança autossômica dominante, de instalação precoce, como disfunção da célula beta pancreática. Mutações heterozigotas em pelo menos sete genes causam MODY. No presente estudo, investigamos a prevalência relativa das mutações da *GCK* (*glucokinase*) e *HNF1α* (*hepatocyte nuclear factor* 1α), as causas mais freqüentes de MODY, em 13 famílias sul-brasileiras com múltiplos casos de diabetes consistentes com MODY. Variantes heterozigotas nos genes da *GCK* e *HNF1α* foram observadas, respectivamente, em uma (7,7%) e em seis (46,2%) famílias. As seis variantes do *HNF1α* provavelmente causaram o diabetes nas famílias nas quais foram observadas. No entanto, não se pode afirmar que a variante GCK Gly117Ser encontrada em uma família seja a mutação causal. Em conclusão, confirmamos que, em uma população do sul do Brasil, as mutações *HNF1α* são uma causa comum de diabetes monogênico em adultos selecionados com critérios clínicos diagnósticos estritos. **(Arq Bras Endocrinol Metab 2008; 52/8:1326-1331)**

Keywords: *Maturity-onset diabetes of the young*; Diabetes monogênico; Fator de transcrição; Glucoquinase

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance, early age of onset (usually before 25 years of age), and pancreatic beta cell dysfunction (1-3).

MODY is not a single entity, but presents genetic as well as metabolic and clinical heterogeneity. Heterozygous mutations in at least seven genes cause MODY (1,4). These genes encode the enzyme glucokinase (*GCK*: MODY2), the transcription factors Hepatocyte Nuclear Factor 4 α (HNF4α: MODY1), Hepatocyte Nuclear Factor 1 α (*HNF1*β: MODY3), pancreatic and duodenal homeobox 1 gene (PDX1: MODY4), Hepatocyte Nuclear Factor 1 beta (*HNF1*β or TCF2: MODY5) neurogenic differentiation 1 (*NEUROD1*) and the insulin gene (*INS*).

MODY2 and MODY3 subtypes may account for up to 70% of cases of MODY in series with strictly defined phenotype (5-7) and MODY1, when tested, might represent a few percent of the cases (8). MODY5 is now recognized to be a multisystemic disorder, with a much wider and heterogeneous clinical spectrum that often does not include diabetes (9,10). Other MODY sub-types are rare disorders, described only in a few families while the prevalence of additional unidentified locus or loci (MODY-X) remains unclear.

The aim of the present study was to investigate the relative prevalence of *GCK* and *HNF1*α mutations in a collection of South-Brazilian families with multiple cases of diabetes consistent clinically with MODY, and to describe their clinical and metabolic profile.

SUBJECTS AND METHODS

Probands of thirteen families were studied. These subjects were identified in the adult outpatient clinics and Diabetes Department of Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul (UFR-GS), Porto Alegre, Brazil. All subjects had overt diabetes and a family history of diabetes in at least two consecutive generations (80% in three generations), with diabetes diagnosed before the age of 25 in at least one individual, and negative pancreatic antibodies. Four additional members of three families were also studied. The research protocol was approved by the local ethics committee, and all patients signed an informed consent form.

Patient evaluation

Patients underwent a standardized evaluation consisting of a questionnaire, physical examination, and laboratory tests. Weight without shoes and in light outdoor clothes and height were measured and body mass index (BMI) was calculated. Measurements of waist circumference at the narrowest point as viewed from the front were performed. Hypertension was defined using the mean of two office blood pressure measurements ≥140/90 mmHg or by the use of antihypertensive medication. An ophthalmologist performed direct fundoscopy, and the presence of diabetic retinopathy was classified as absent, non-proliferative, or proliferative. Patients were classified into normoalbuminuric (urinary albumin excretion [UAE] <30 mg/24 h), microalbuminuric (UAE 30–300 mg/24 h), or macroalbuminuric (UAE ≥300 mg/24 h), based on 24-h sterile urine collections, confirmed in two out of three samples. Ischemic heart disease was established in the presence of angina or possible infarct according to the World Health Organization Cardiovascular Questionnaire and/or the presence of resting electrocardiogram abnormalities. Neurological tests were performed to evaluate sensation, deep reflexes, and strength.

Laboratory methods

UAE was measured by immunoturbidimetry (Microalb; Ames-Bayer, Tarrytown, NY; intra- and interassay coefficients variation of 4.5 and 11.0%, respectively). HbA1c was measured by a high-performance liquid chromatography system (normal range 4-6%; Merck-Hitachi 9100). Fasting plasma glucose was measured by the glucose-peroxidase colorimetric enzymatic method (Biodiagnóstica). Creatinine was measured by the Jaffé method and the lipid profile by a colorimetric method. Serum C-peptide was measured by chemiluminescence, glutamic acid decarboxylase (GAD) antibody by radioimmunoassay (11).

Molecular analysis

The promoter, the coding regions and intron-exon junctions of *GCK* and *HNF1*α were screened for mutations by direct sequencing.

Statistical analysis

Student's t test or X^2 tests were used to compare clinical and laboratorial data. Data are expressed as the means ± SD. Variables without normal distribution were expressed as median (range) and were log-transformed for statistical tests. P values <0.05 (two tailed) were considered to be significant.

RESULTS

Thirteen families with clinically defined MODY were screened (Figure 1). Heterozygous variants in glucokinase and *HNF1α* genes were observed, respectively, in one (7.7%) and six (46.2%) families (Table 1). A c.349G>A variant in exon 3 of the glucokinase gene that results in the replacement of a glycine by a serine at codon 117 (p.Gly117Ser) was observed in the proband from kindred FER, but was not present in the proband's elder sister who also had diabetes. No other *GCK* variant was observed in these families.

Table 1. Allelic variations in MODY genes.

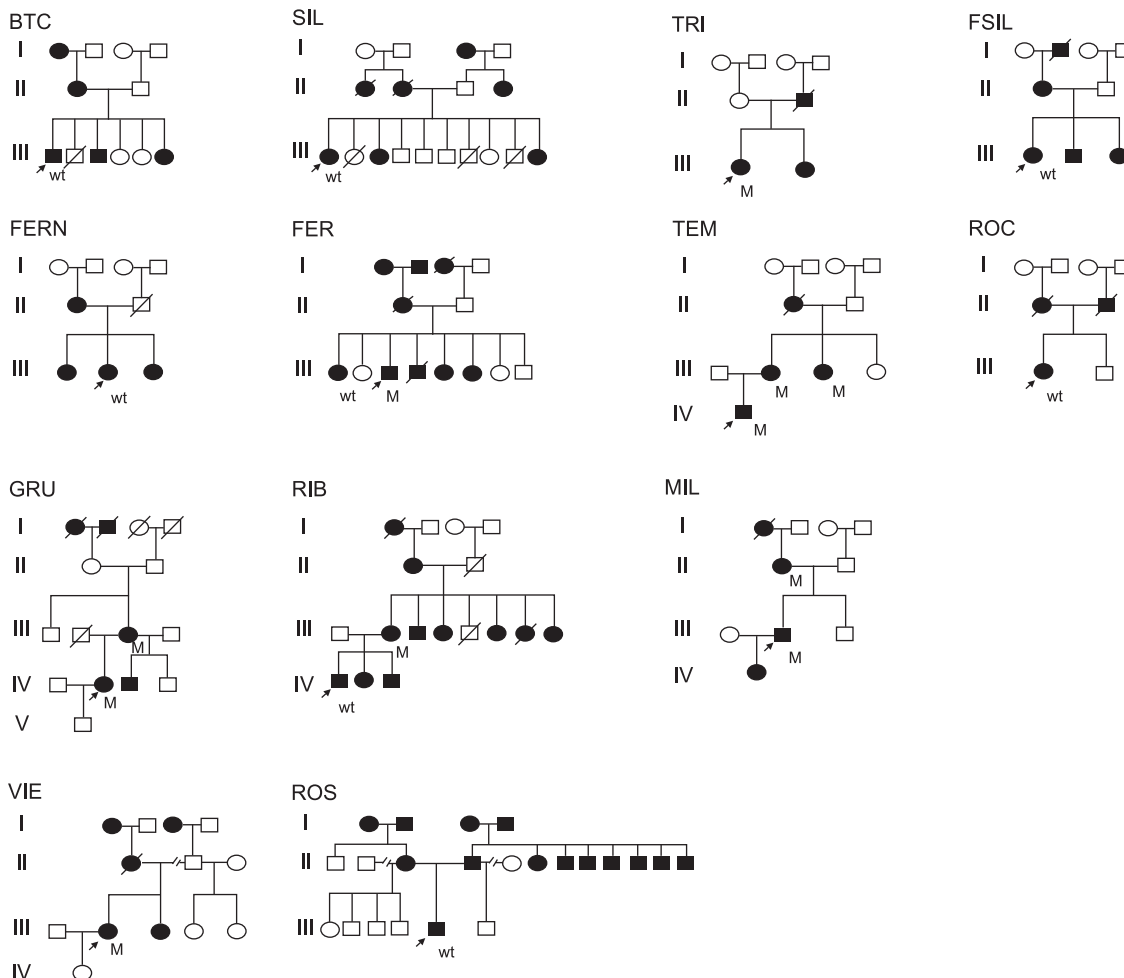| Gene	Location	Nucleotide change	Protein change	Kindred
GCK	Exon 3	c.349G>A	p.Gly117Ser	FER
HNF1α	Exon 1	c.24_35dup12	p.Gln9_Leu12dup	MIL
HNF1α	Exon 1	c.92G>A	p.Gly31Asp	RIB
HNF1α	Exon 3	c.638T>C	p.Ile213Thr	TEM
HNF1α	Exon 4	c.872_873insC	p.Gly292Arg fsX24	TRI
HNF1α	Exon 6	c.1136_1137 delCT	p.Pro379Arg fsX37	GRU
HNF1α	intron 7	c.1501+1G>T	unknown	VIE

Figure 1. Pedigrees of the 13 families. Arrows identify the probands. Squares denote male family members, and circles denote female family members. Wt stand for wild type and M for mutant alleles of glucokinase or HNF-1α genes.

A duplication of 12 nucleotides (c.24_35dup12: GCAGACGGAGCT) in exon 1 of *HNF1α* resulting in the duplication of four aminoacids (Gln, Thr, Glu, Leu) was observed in the proband from kindred MIL as well as in his diabetic mother. Two missense variants were also observed. A c.92G>A variant in exon 1 leading to the replacement of a glycine by an aspartic acid at codon 31 (p.Gly31Asp) was found in the proband from kindred RIB, but not in his diabetic son. A c.638T>C variant in exon 3, that results in the replacement of a isoleucine by a threonine at codon 213 (p.Ile213Thr) was observed in the proband from kindred TEM and in two other diabetic family members. The insertion of a cytosine at position c.872_873 in exon 4 resulting in a frameshift with a stop codon at position 24 of the new reading frame (p.Gly292Arg fsX24) was observed in the proband of kindred TRI. A deletion of two nucleotides at position c.1136_1137 (CT) in exon 6 leading to a frameshift with a stop codon at position 37 of the new reading frame (p. Pro379Arg fsX37) was observed in the proband of kindred GRU. Finally, a c.1501+1G>T replacement in the first base of the splice donor site of intron 7 was observed in the proband of kindred VIE.

Demographic and clinical profile of MODY3 subjects and subjects negative for MODY3 mutations are shown in Table 2. Except for a younger age at diagnosis in MODY3 subjects, no differences were observed between the two groups. All participants had negative antiGAD antibodies.

DISCUSSION

We have detected one variant in the glucokinase gene and six different variants in the *HNF1α* gene in seven families of a panel of thirteen Brazilian kindred, with clinical diagnosis of MODY. It is unclear whether the glucokinase Gly117Ser variant observed in the proband of family FER is a causal mutation. The variant was not observed in the proband's elder sister who had diabetes diagnosed at the age of 16. Moreover, the proband is overweight and her sister is obese (BMI of 29.1 and 30.6 kg/m², respectively), which is uncommon in subjects with *GCK*-related diabetes. The glycine at position 117 is predicted to be potentially important for glucose phosphorylation, but both glycine and serine are neutral and polar aminoacids. In vitro studies are needed to assess the functional impact of this variant.

Table 2. Clinical and laboratory characteristics of MODY3 and MODY X patients.

	MODY 3 (N= 10)	MODY X (N = 06)	p
Male/female	2/8	1/5	0.69
Age at diagnosis (years)	21 ± 5	32 ± 13	0.029
Body mass index (kg/m²)	24 ± 3	24 ± 4	0.68
Male waist (cm)	95 ± 5	102 ± 6	0.23
Female waist (cm)	84 ± 8	76 ± 11	0.22
Hypertension n (%)	2 (20)	2 (33.3)	0.49
Total Cholesterol (mg/dl)	197 ± 37	195 ± 61	0.95
HDL Cholesterol (mg/dl)	58 ± 7.4	51 ± 22	0.56
Triglycerides (mg/dl)	81 (41-130)	93 (56-580)	0.35
FPG (mg/dl)	125 ± 49	152 ± 71	0.49
HbA1c (%)	7.06 ± 1.6	7.5 ± 2.1	0.67
Nephropathy n (%)	2 (20.0)	1 (16.7)	0.20
Neuropathy n (%)	1 (10.0)	1 (16.7)	0.64
Retinopathy n (%)	4 (40.0)	2 (33.3)	0.55
Heart disease n (%)	3 (30.0)	1 (16.7)	0.50

Data expressed as mean±SD, number of cases (%) or median (range); FGP: fasting plasma glucose.

The *HNF1α* variants are likely to cause diabetes in the families where they were observed. The p.Ile213Thr cosegregates with diabetes in family TEM and is predicted to be deleterious to *HNF1*A function (Polyphen, SIFT, Panther and SNPs3D prediction models). The p.Gly31Asp observed in the proband from family RIB was not observed in her son who also had diabetes, but was previously found to cosegregate with diabetes in a French MODY family (5). The two frameshift mutations (p.Gly292Arg fsX24, p.Pro379Arg fsX37) generate truncated proteins that probably present severely impaired transcriptional activity. Moreover, the p.Gly292Arg fsX24 was previously found to cosegregate with diabetes in a MODY family from Scotland (12). The insertion in intron 7 (c.1501+1G>T) abolishes the splice donor site resulting almost certainly in the generation of abnormal transcripts. The duplication of four aminoacids in exon 1 (p.Gln9_Leu12dup) seems to cosegregate with diabetes in family MIL.

Mutations in the *HNF1α* gene are the most frequent cause of monogenic diabetes in adults. It was estimated that they may account for approximately 1% of cases of diabetes (13). More than 300 different mutations have been reported to date (14,15). Studies in several populations (5-7,16-19), including two Brazilian studies (17,19), have shown that *GCK* and *HNF1α* mutations account to about half the cases of clinically defined MODY (familiar diabetes with early age of onset). When the autosomal dominant transmission can be clearly ascertained in families within three generations and the phenotype is strictly defined, the frequency of observed *GCK* and *HNF1α* mutations may be higher still (5,7). Our findings are, therefore, in accordance with reported series. A recurrent question at the end of these studies, including ours, is regarding the etiology of diabetes in *GCK*, *HNF1α*, HNF4α-negative cases. Possible explanations would include mutations in other unknown genes causing monogenic diabetes, combination of rare polymorphisms with a minor effect in known MODY genes, other molecular defects of *GCK* or *HNF1α* not detected by sequencing (20) and polygenic type 2 diabetes with early onset.

Making a diagnosis of MODY subtypes and other monogenic forms of diabetes is important for appropriate patient care, as well as being of scientific interest. Patients with *GCK*, *HNF1α*/HNF4α or *HNF1*B mutations have different clinical courses, regarding the progression of hyperglycemia, treatment requirements, complication risk and pregnancy outcome (1,9,14,21-24).

Recently, guidelines have been developed to suggest clinical criteria for the molecular testing of monogenic diabetes (25,26). Thus, diabetes in the presence of extrapancreatic features such as renal cysts and genitourinary malformations points out to *HNF1*B mutations (9), and in the presence of deafness and pigmentary retinopathy suggest the mitochondrial m.3243A>G mutation (27). A mild and relatively stable chronic hyperglycemia that increases little after food or an oral glucose load, with very early age of onset (usually diagnosed at birth or in early childhood), without complications and for which treatment is rarely needed, strongly suggests a *GCK* mutation (23). Testing for *HNF1α*/HNF4α mutations is recommended in patients with young-onset diabetes (<25 years in at least one family member), family history of diabetes in at least two generations, absence of pancreatic islet autoantibodies, glycosuria at blood glucose levels <180 mg/dl (low renal threshold), marked sensitivity to sulfonylurea, and no marked obesity or evidence of insulin resistance.

In conclusion, we have confirmed in a South-Brazilian population that *HNF1α* mutations are a common cause of monogenic diabetes in adults selected with strict clinical diagnostic criteria. *HNF1α* mutations were identified in half of the patients with a family history of diabetes in three consecutive generations, in which at least one individual had diabetes diagnosed before the age of 25. Subjects with *HNF1α* mutations had a diagnosis of diabetes at a younger age than subjects without mutations, but our study did not have the power to detect other clinical and biological differences between the two groups of subjects. It is now clear that there are differences in prognosis and treatment of the various forms of monogenic diabetes. They strongly support the use of molecular genetic testing for a precise diagnosis.

No potential conflict of interest relevant to this article was reported.

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