



Short Communication

## Variants of the *HNF1 $\alpha$* gene: A molecular approach concerning diabetic patients from southern Brazil

Naieli Bonatto<sup>1</sup>, Viviane Nogaroto<sup>2</sup>, Paulo V. Svidnicki<sup>2</sup>, Fábio Q. Milléo<sup>3</sup>, Sabrina Grassioli<sup>4</sup>,  
Mara C. Almeida<sup>2</sup>, Marcelo R. Vicari<sup>2</sup> and Roberto F. Artoni<sup>2</sup>

<sup>1</sup>Programa de Pós-Graduação em Genética, Departamento de Genética, Universidade Federal do Paraná, Curitiba, PR, Brazil.

<sup>2</sup>Programa de Pós-Graduação em Biologia Evolutiva, Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil.

<sup>3</sup>Departamento de Cirurgia, Hospital Vicentino da Sociedade Beneficente São Camilo, Ponta Grossa, PR, Brazil.

<sup>4</sup>Programa de Pós-Graduação em Biologia Evolutiva, Departamento de Biologia Geral, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil.

### Abstract

Maturity Onset Diabetes of the Young (MODY) presents monogenic inheritance and mutation factors which have already been identified in six different genes. Given the wide molecular variation present in the hepatocyte nuclear factor-1 $\alpha$  gene (*HNF1 $\alpha$* ) MODY3, the aim of this study was to amplify and sequence the coding regions of this gene in seven patients from the Campos Gerais region, Paraná State, Brazil, presenting clinical MODY3 features. Besides the synonymous variations, A15A, L17L, Q141Q, G288G and T515T, two missense mutations, I27L and A98V, were also detected. Clinical and laboratory data obtained from patients were compared with the molecular findings, including the I27L polymorphism that was revealed in some overweight/obese diabetic patients of this study, this corroborating with the literature. We found certain DNA variations that could explain the hyperglycemic phenotype of the patients.

**Key words:** MODY3, molecular diagnosis, diabetes mellitus, nucleotide sequencing.

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Maturity Onset Diabetes of the Young (MODY), which comprises between 1 to 5% of all the cases of diabetes, is characterized by monogenic autosomal dominant inheritance, early onset (usually before 25 years of age), with at least one and ideally two family members affected, and the dysfunction of pancreatic  $\beta$  cells (Tattersall, 1974; Velho and Froguel, 1998). The six well-characterized subtypes of MODY that are related to mutations in six different genes are, *GCK*, which encodes the glucokinase enzyme (MODY2) (Froguel *et al.*, 1992) and five for transcription factors, such as *HNF4 $\alpha$*  (MODY1) (Yamagata *et al.*, 1996a), *HNF1 $\alpha$*  (MODY3) (Yamagata *et al.*, 1996b), *IPF1* (MODY4) (Stoffers *et al.*, 1997), *HNF1 $\beta$*  (MODY5) (Hori-kawa *et al.*, 1997) and *NeuroD1/ $\beta$  2* (MODY6) (Malecki *et al.*, 1999). MODY2 and MODY3 are the most common sub-types, with frequencies that vary according to the pop-

ulation. In Brazil, the prevalence of MODY3 is 13%-46.2%, followed by MODY2 with 7.7%-12.5% (Moises *et al.*, 2001; Furuzawa *et al.*, 2008). Furthermore, Maraschin *et al.* (2008) suggested that the majority MODY cases in Brazil are due to MODY-X genes. These MODY sub-types are rare disorders identified in some families, while the locus involved (called MODY-X) has not yet elucidated (Maraschin *et al.*, 2008).

The accurate and early diagnosis of diabetes can be decisive in the clinical management of less severe cases, as, for example, MODY2 (non-progressive and with a low prevalence of microvascular complications) (Froguel *et al.*, 1993), but especially in situations of major health problems as in the case of MODY3 (Yamagata *et al.*, 1996b). Therefore, this study aimed at exploring possible mutations related to *HNF1 $\alpha$*  in patients clinically diagnosed as MODY diabetes, from localities in the Campos Gerais region, Paraná State, in southern Brazil. This study sought to relate the clinical profile of these patients with their molecular characterization. The *HNF1 $\alpha$*  gene (MODY3) was chosen for investigation. The other MODY sub-types were not tested,

due to their rare incidence in Brazilian populations (Moises *et al.*, 2001; Furuzawa *et al.*, 2008).

This research was approved by the Committee for Ethics in Human Research at the Universidade Estadual de Ponta Grossa (COEP n° 14/2009). The sample consisted of seven unrelated patients suffering from medicinally untreatable diabetes, together the early onset of severe and progressive hyperglycemia, concurrently affecting other family members. According to Ellard *et al.* (2008), MODY diabetes is characterized by monogenic autosomal dominant inheritance, early onset (usually before 25 years of age), with at least one and ideally two, family members affected, a family history of diabetes (at least two generations), the absence of pancreatic islet autoantibodies, non-insulin independence outside the normal honeymoon period (3 years), no insulin resistance, and dysfunction of pancreatic  $\beta$  cells. Some of the clinical characteristics of these patients can be seen in Table 1.

Genomic DNA was extracted from blood samples with commercial kits (Qiagen). PCR (Polymerase Chain Reaction) amplification was with oligonucleotides for the flanking regions of 10 exons of the *HNF1 $\alpha$*  gene (Nogaroto *et al.*, 2011). Following electrophoresis, the samples were purified using commercial kits (Roche), and then sequenced in an automatic ABI-PRISM 3100 sequencer (Applied Biosystems). Molecular analysis of the amplified

fragments revealed seven variations in the *HNF1 $\alpha$*  gene, five synonymous and two missense mutations (Table 2).

Generally, silent mutations are usually classified as allelic polymorphisms, which are discarded in analyses of wider interest because they are considered to be neutral. Cartegni *et al.* (2002) compiled more than 20 studies reporting specific points of synonymous mutations within coding regions associated with altered splicing, which in turn led to the exclusion of certain exons. In this study, none of the synonymous variants found in exons of the *HNF1 $\alpha$*  gene was associated with known donor sites for splicing.

*HNF1 $\alpha$*  protein essentially consists of three functional domains: the dimerization domain (N-terminal), the DNA binding domain (with a POU<sub>S</sub> motif and a POU<sub>H</sub> homeodomain region) and the transactivation domain (C-terminal) (Ryffel, 2001). The missense mutations found in the present study are contained in the dimerization domain of the protein (I27L), and in the POU DNA binding domain (A98V). Whereas, on studying patients bearing this allelic variation, Chiu *et al.* (2003) found a moderate risk of developing type 2 diabetes mellitus (T2DM), and an increased risk was reported by Chen *et al.* (2010). *In vivo*, the A98V polymorphism demonstrated a deterioration in insulin release in response to glucose over time, whereas the I27L was associated with a propensity to develop T2DM, especially in 60-plus-year-old overweight individuals (Holmk-

**Table 1** - Some clinical features of the patients included in this study.

Data	Patients						
	1	2	3	4	5	6	7
Gender	Female	Female	Male	Male	Female	Female	Male
Age/age of diagnosis	60/46	50/36	40/26	48/38	39/16	48/40	60/45
Number of affected relatives, degree of kinship between brackets	3 (1)	1 (1)	1 (1)	1 (1)	4 (1)	2 (1)	4 (1)
BMI (kg/m <sup>2</sup> )	28.37	30.30	26.47	33.57	33.30	28.58	31.94
Hypertension	Yes	Yes	No	No	Yes	No	Yes
Diabetes treatment	Insulin	Insulin	Insulin	Oral agent	Insulin	Oral agent	Insulin + oral agent
Micro/macrovascular complications	Yes/yes	No/no	No/no	No/no	Yes/yes	No/yes	Yes/no
HbA <sub>1c</sub> (%)	6.40	14.30	9.78	10.68	11.30	11.45	12.60
Fasting glycemia (mg/dL)	165	281	406	278	260	305	301
Postprandial glycemia (mg/dL)	186	364	332	420	398	326	324
C peptide (ng/mL)	2.50	3.37	1.40	3.50	1.20	2.10	1.20
Basal insulin ( $\mu$ UI/mL)	5.0	11.3	9.3	13.8	28.3	9.1	20.3
Postprandial insulin ( $\mu$ UI/mL)	17.0	45.6	16.5	15.7	12.4	12.0	9.8
Anti-glutamic acid decarboxylase antibodies ( $\mu$ U/mL)	0.5	0.5	0.2	0.2	0.1	0.5	0.4
Anti-Langerhans islets antibody	No reagent	No reagent	0.8	No reagent	0.2	0.5	0.6
Anti-insulin antibody (U/mL)	1.0	3.20	0.80	1.60	2.40	0.61	0.50

Reference values: BMI (Body Mass Index): 18.5-24.9 kg/m<sup>2</sup> (normal weight), overweight (25-29.9 kg/m<sup>2</sup>), mild obesity – grade 1 (30-34.9 kg/m<sup>2</sup>), according to World Health Organization (1998); HbA<sub>1c</sub>: 4.0-7.0%; Fasting glycemia:  $\leq$  100 mg/dL; Postprandial glycemia (2-hour post challenge load):  $<$  140 mg/dL; C peptide: 1.1 a 5.0 ng/mL; Basal insulin: = 29.1  $\mu$ UI/mL; Postprandial insulin:  $<$  150  $\mu$ UI/mL; Anti-glutamic acid decarboxylase antibodies:  $<$  1.0U/mL; Anti-Langerhans Islets antibody: no reagent; Anti-insulin antibody: = 1 U/mL; Hypertension: arterial pressure 130/80 mm Hg.

**Table 2** - Variations found in the *HNF1α* gene through nucleotide sequence analysis.

Patients	Localization	Codon	Nucleotide change	Aminoacid change	Reference
5	Exon 1	15	CTCCTG	A15A	1
1, 2, 5, 7	Exon 1	17	GCCGCA	L17L	2
1, 2, 5	Exon 1	27	ATCCTC	I27L	2
1	Exon 1	98	GCCGTC	A98V	2
6	Exon 2	141	CAGCAA	Q141Q	1
2, 3, 5	Exon 4	288	GGGGGC	G288G	3
2	Exon 8	515	ACGACA	T515T	4

Legend: (1) present paper, (2) Yamagata *et al.* (1996b), (3) Yang *et al.* (2006), (4) Jafar-Mohammadi *et al.* (2009).

vist *et al.*, 2006). This relationship between BMI and I27L was also reported by Ranade *et al.* (2010), where 80% of the patients with this variant were also overweight. These data are consistent with our findings, wherein two of the three patients harboring the polymorphism were obese and one was overweight.

In Scandinavian carriers of the A98V allelic variation, Holmkvist *et al.* (2006), assumed a significant and progressive reduction in the secretion of insulin before glucose ingestion, whereas Lehto *et al.* (1999) proposed an association between this polymorphism and the early onset of T2DM. This has also been observed in patients from India (Anuradha *et al.*, 2005). Rissanen *et al.* (2000) found an association between the 98V allele and late onset T2DM in Finnish patients, but not in Chinese. Anuradha *et al.* (2005) correlated A98V and the early onset MODY type diabetes in Indians. Increased frequency of A98V polymorphism was noted in a sample of Brazilian patients, with late-onset autosomal dominant type diabetes mellitus (Giuffrida *et al.*, 2009). As regards MODY3, the main variants to be found in the *HNF1α* gene are I27L, A98V, G319S and S487N (Holmkvist *et al.*, 2006). Worthy of note: the lack of consensus in identifying some DNA variations as being present in T2DM or monogenic diabetes (MODY), hampers, not only in the correct diagnosis of which type of diabetes the patient has, but also in discriminating what would be relevant for its molecular characterization and treatment.

The severe failure of glycemic control in patients, placed in evidence by fasting and postprandial testing, is compatible with the failure of insulin secretion in response to glucose, typical of MODY3 patients (Glamočlija and Jevric-Causevic, 2010). The marked presence of polymorphisms, already associated with T2DM, but also present in the gene responsible for MODY3, permits questioning the classification of these patients as type 2 diabetic patients or as typical MODY3 patients. Factors, such as obesity and insulin resistance, are requisites for triggering the onset of diabetes (Hegele *et al.*, 1999). Furthermore, the perceptibly constant presence of overweight patients in this study could be an indication of an even more complex relationship between the development of obesity and the polymorphism found, especially as regards patient 1, who carried both the variants I27L and A98V, and who, at the age of 41, was

overweight and presented hypertension and micro and macrovascular complications.

In conclusion, we found certain DNA variations that could explain the hyperglycemic phenotype of the patients. This study found variations in exonic sequences for the *HNF1α* gene in the patients corresponding to five silent mutations, in addition to the variants I27L and A98V, which have previously been described in patients with the common form of MODY and T2DM, but were also found in non-diabetic patients (Hegele *et al.*, 1999; Rissanen *et al.*, 2000; Giuffrida *et al.*, 2009), thereby reflecting their controversial significance variations. Thus, different population studies also reported different conclusions about the molecular findings of *HNF1α*, sometimes linking them to completely different types of diabetes, including those of monogenic or multifactorial origin. A consensus concerning this scenario should be discussed in future studies, with a mind to facilitating the correct classification of the polymorphisms found, thereby leading to more accurate diagnosis of diabetes types. The regulation of expression of these genes in diabetic patients with these allelic variations could be explained in part by epigenetic differences, as well as by environmental factors, resulting in a complex and still open issue.

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