Variants of the \( HNF1\alpha \) gene: A molecular approach concerning diabetic patients from southern Brazil

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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\(\alpha\) (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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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 β 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 HNF1α 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 HNF1α 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 HNF1α gene was associated with known donor sites for splicing.

HNF1α protein essentially consists of three functional domains: the dimerization domain (N-terminal), the DNA binding domain (with a POU5 motif and a POUH 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 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-
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 (Glamoclija 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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