MUTATIONS IN THE GCK GENE PROMOTER MAY BE RESPONSIBLE FOR MODY2 DISEASE

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Abstract

According to the World Health Organization there are at least 346 million people worldwide suffering from some form of diabetes. Besides diabetes mellitus (DM) types 1 and 2, today a large number of gene mutations also result in hyperglycemia, causing monogenic DM. Maturity-onset diabetes of the young (MODY) is part of a heterogeneous group of DM caused by a single gene mutation and is characterized by autosomal dominant inheritance and primary defects of insulin secretion of pancreatic beta cells and non-insulin-dependent DM with a young age at diagnosis. MODY may account for up to 5% of all cases of DM, which are often not diagnosed or are misclassified. MODY is linked with mutations within 13 different genes. Among these, mutations in the glucokinase gene can cause MODY2. More than 600 SNPs have been described in this gene, including those in intron regions. This study was performed with 3 volunteers from the same household with a MODY2 diagnosis. We propose to identify the occurrence of mutations in the GCK gene by sequencing DNA of the volunteers. Mutations in the GCK gene were observed and we report three new mutations in the promoter gene, never described before.

Introduction

Diabetes is among the major metabolic diseases affecting millions of people worldwide. Estimates indicate a kind of epidemic with more than 170 million individuals worldwide suffering from some form of diabetes, a prevalence of 2.8%. This epidemic is growing fast, and the prevalence is projected to double by 2025 (1, 2). In Brazil, the situation is the same. According to the WHO, the country had around 4.6 million diabetics in 2000 and this number could reach 11.3 million in 2030 (3).

Traditionally, diabetes mellitus (DM) was classified according to the age of symptom onset: juvenile diabetes or adult onset diabetes. However, in 1979, the National Diabetes Data Group from the National Institutes of Health proposed a classification based on the therapeutic needs of the patient: DM-insulin-dependent or non-insulin-dependent. In 1997, due to the accumulation of knowledge on the subject, a new classification was recommended, based on the etiology and pathogenesis of diabetes (4). Type 1 DM, a cellular-mediated autoimmune destruction of the β-cells of the pancreas results in a deficiency of insulin secretion; Type 2 DM, heterogenic etiopathogeny leads to
a combination of resistance to insulin action and an inadequate compensatory insulin secretory response; a third
group, known as other types of diabetes, including genetic defects that affect the function of β-cells, diseases that
diffusely injure the pancreas, such as pancreatitis, trauma, endocrinopathies, drugs that impair insulin secretion,
preferring diabetes in individuals with insulin resistance, infections caused by certain viruses that induce β cell
destruction, and others; and a fourth group that includes gestational diabetes (GDM), characterized by glucose
intolerance of variable severity that begins or is first diagnosed during pregnancy and usually resolves not long after
delivery (5, 6).

In the third group, solid research on the monogenic forms known as MODY (Maturity-Onset Diabetes of
the Young) contributed greatly to the area of clinical endocrinology, mainly for diagnosis of patients with complex
frameworks symptoms, cases in which conclusive diagnostic is usually very difficult. It is estimated that monogenic
forms account for some 2–5% of all cases of DM (7). MODY is typically diagnosed before 25 years of age, is a
dominant form of diabetes, not ketogenic and non-insulin dependent, with high penetrance and autosomal dominant
transmission (8). This is a heterogeneous group of diabetes because the symptoms may be caused by mutations in
different genes, known as MODY genes (9). So far, the phenotype of MODY is associated with mutations in 13
different genes: HNF4A (MODY1), GCK (MODY2), HNF1A (MODY3), Pdx1 (MODY4), HNF1B (MODY5),
NEUROD1 (MODY6), KLF11 (MODY7), CEL (MODY8), PAX4 (MODY9), INS (MODY10), BLK (MODY11)
ABCC8 (MODY12) and KCNJ11 (MODY13) (10).

These different types of monogenic diabetes have similar characteristics: early diagnosis, usually between
10 and 45 years of age; family history of diabetes, in every generation, due to autosomal dominant inheritance;
blood of obesity; usually mild hyperglycemia, without the need for insulin therapy; negative tests for the presence
of auto antibodies (GAD 65 and IA2) against pancreatic beta cells; and irregular insulin secretion by pancreatic β
cell (9).

There is no consensus on prevalence of MODY thus far. Costa and colleagues in 2000 (11) showed that the
prevalence of different subtypes of MODY vary widely in studies of populations in England, France and Germany.
However, MODY 2 seems to be one of the most frequent types of MODY, ranging from 8 to 63% of cases (11, 12,
13). MODY is more common in some communities in the world, as in Pima Indians, the Nauru population and more
recently in Asian Indians in South Africa and Southern India (14). Another interesting aspect of epidemiology is the
low prevalence in blacks, and high prevalence in Caucasians (15). According to Moises and colleagues in 2001 (16)
and Furuzawa and colleagues in 2008 in Brazil, the prevalence of MODY2 ranges from 7.7% to 12.5%.

MODY2 is associated with mutations in the gene encoding glucokinase (GCK), which is part of the family
of hexokinase enzymes. GCK has an important regulatory role in beta cells and catalyzes the first reaction in the
glycolytic pathway converting glucose to glucose-6-phosphate (G6P) and thus has a crucial role in the regulation of
insulin secretion (18). Prognosis of MODY2 is better than other types, since hyperglycemia is mild; the disease is
not progressive; does not require treatment, and usually does not have secondary complications (19, 20).

It is believed that the MODY represents 2-5% of all cases of DM, and the biggest problem is that often
these cases are not diagnosed or are misclassified as type 1 or 2 DM (9). Furthermore, a specific diagnosis of
MODY is important in guiding appropriate treatment, avoiding future micro and macrovascular complications, as
well as in helping to identify the risk of diabetes in other family members. For this reason, the aim of this work is to
identify the occurrence of mutations in the GCK gene in patients with clinical diagnosis of MODY2.

Materials and methods

Subjects

We selected and included 3 related Brazilian volunteers in this study, with a history of impaired fasting
hyperglycemia or impaired glucose tolerance. Informed consent (IC) was obtained from all participants or from a
responsible parent and was approved by the ethics committee from our institution (ref number IBUSP 113/210 - FR.
387425).

Sample collection, DNA extraction and sequencing

The samples were collected by buccal swab and the total DNA was extracted using a standard protocol as
reported before (21). The primers used in this study were adapted from Boutin and colleagues in 2001 (22). The
PCR was performed by Go®Taq Flexi DNA Polymerase kit (Promega, Madison, WI, USA) following the
manufacturer’s instructions. The amplicons were cloned with the TopoTA Cloning® kit (InvitrogenTM by Life Technologies, Foster City, CA, USA). Plasmidial DNA was prepared by the alkaline lyses method (Sambrook & Russel, 2001) and the sequencing was carried out using the Big Dye® Terminator v3.1 Cycle Sequencing (Applied Biosystems™ by Life Technologies, Foster City, CA, USA) and determined in an ABI 3500XL instrument (Applied Biosystems™) according to the manufacturer’s instructions. The sequences were analyzed using Lasergene® SeqMan Pro™ for MacOs, version 11.2.1 (DNASTAR®) and were compared with the GenBank human genomic plus transcript database using the BLAST tool (23).

Results and discussion

In routine laboratory tests, a young woman showed slightly altered blood glucose levels. Additional tests did not indicate the classical types of DM. According to the patient, a history of mild hyperglycemia was common in her family, without the need of insulin therapy. Considering the existence of other cases of mild hyperglycemia in the family in different generations, the absence of insulin, normal laboratory tests (Table 1), with the exception of the glycemic indices, and non-obese individual, we decided to investigate the hypothesis of MODY2 (24). Laboratory tests also indicated changes in glycemic indices of her son (Table 1), thus samples from the mother and her two children were collected for molecular tests (Fig 1.)

![Heredogram of two generations for the family tested in this study. The pedigree of 3 probands is shown. Black boxes represent affected and blank boxes represent unaffected individuals. Only mother and children were tested.](image)

**Table 1. Clinical laboratory findings for probands 1 and 3**

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Proband 1</th>
<th>Proband 3</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of diagnosis</td>
<td>30 yr</td>
<td>5 yr</td>
<td>-</td>
</tr>
<tr>
<td>Insulin therapy</td>
<td>No</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Insulin level</td>
<td>3.7 µIU.ml⁻¹</td>
<td>2.00 µIU.ml⁻¹</td>
<td>&lt; 29.1 µIU.ml⁻¹</td>
</tr>
<tr>
<td>C-peptide</td>
<td>NT</td>
<td>0.60 nmol.l⁻¹</td>
<td>0.9-7.1 nmol.l⁻¹</td>
</tr>
<tr>
<td>GAD antibodies</td>
<td>NT</td>
<td>0.60 U.ml⁻¹</td>
<td>1.00 U.ml⁻¹</td>
</tr>
<tr>
<td>ICAs</td>
<td>NT</td>
<td>0.20 U.ml⁻¹</td>
<td>&lt; 0.40 U.ml⁻¹</td>
</tr>
<tr>
<td>IFCC-HbA1c</td>
<td>49 mmol.mol⁻¹</td>
<td>53 mmol.mol⁻¹</td>
<td>&lt;53 mmol.mol⁻¹</td>
</tr>
<tr>
<td>FBG</td>
<td>5.9 mmol.l⁻¹</td>
<td>6.0 mmol.l⁻¹</td>
<td>3.6-5.8 mmol.l⁻¹</td>
</tr>
</tbody>
</table>

GAD: Glutamic acid decarboxylase; ICAs: islet cell antibodies; IFCC-HbA1c: glycated hemoglobin; FBG: fasting blood glucose, NT – Not tested;

The GCK gene (7p15.3–p15.1) spans approximately 45,169 bp, and its 10-exon processed mRNA encodes a 465-amino-acid protein (32). Since the first correlation between the GCK gene and NIDDM (25, 26), more than 600 SNPs were described already in the GCK gene; nevertheless, most of them do not affect the glucokinase structure or function (27). Related promoter mutations are clearly the least reported. The -71G>C (28) and -30 (29) are well known promoter mutations associated with the risk of gestational and type 2 DM or MODY2, respectively. In our study, both regions are in their “wild-type” forms (Fig. 2).

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However, what can explain the mild hyperglycemia, without other genetic evidence, if the hypothesis of MODY 2 is correct? In this study, we report three new mutations on the region of the GCK gene’s first promoter. The data showed all of these were transition C>T (-610, -492 and -261) located in the rich GC content site (Fig. 2).
Transition C>T is a common mutagenic mechanism resulting from a deamination of methylated cytosine (5-meC) (30) which in latest analysis, corroborate the hypothesis of epigenetic events associated with the gene expression deregulation causing MODY and type 2 diabetes (31).

These findings led to the hypothesis that the three SNPs located within the region of the same promoter could change the expression pattern of the GCK gene, having not only genetic, but also metabolic and physiological implications. In this work we showed evidence of epigenetic events in the promoter of this important gene for the metabolism of glucose. The epigenetic pathway controlling the GCK gene expression is not fully understood; however, further studies in this field may reveal consequent mutagenic processes, eventually with an evolutionary meaning.

From this point, our findings make a significant contribution to future studies of MODY2 and implications of non-coding sequences and epigenetic regulation to the correct functioning of the transcription machinery of the GCK gene. Our intention is also to initiate evolutive studies correlating patterns of epigenetic inheritance, mutagenesis and risk of the MODY disease.

The study is still running and additional volunteers from the same family will be invited to participate. Complementary tests are being evaluated such as relative quantification at mRNA level and electrophoretic mobility shift assay.

**Conclusion**

In this study we report three new C>T mutations in the region of the GCK gene promoter. This is the first study that reports this number of SNPs in that region. The findings led us to hypothesize an alteration of the normal expression pattern of gene GCK, resulting in the mild hyperglycemia shown in the proband.
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References

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