GCK gene mutational analysis and MODY2 monogenic diabetes

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Introduction

Diabetes mellitus is a metabolic disorder multiple etiology, characterized by the presence of high levels of blood glucose (chronic hyperglycemia), with disorders in the metabolism of carbohydrates, fats and proteins. It includes diabetes mellitus type 1 and type 2. Type 1 diabetes mellitus results from serious effects on the health of patients, and develop long-term damage, with dysfunction of various organs. In this context it can lead to serious consequences resulting from the development of any form of diabetes, it is particularly careful the knowledge of the causes that lead to the development of the disease. The present study, conducted on two levels, molecular and clinical, focuses on the identification of the causes that determine the onset of the MODY2 monogenic diabetes, by the search of different in these three district is the expression of one isoform rather than another. The aim of this research was to identify in a specific group of patients, suffering from hyperglycemia, the presence of mutations in the GCK gene, to understand, at molecular level, how the structure, the regulation and the catalysis of the enzyme are altered and if such mutations could be a possible cause of the diagnosis of MODY2 monogenic diabetes. The latter case is particular important for a clinical point of view, because since it derives the therapeutic choice whether to treat those who are positive or negative after the results of the screening, with particular attention to pregnant women. Generally, MODY2 does not need insulin therapy, but it needs only a dietary treatment, with some exceptions during pregnancy, where it would be important to know fetal genotype. In fact, gestational diabetes is closely associated with MODY2 diabetes, because GCK gene mutations may influence the expression of different isoforms of the enzyme. In about 6% of the cases, gestation diabetes is the first clinical manifestation of MODY2, of which you are aware of being affected, thanks to pregnancy. This link justifies the choice of the two study groups of research: the subject with diabetes’ onset under 25 years and women with gestational diabetes.

Monogenic diabetes represents all the form of diabetes caused by a single mutation in a single and specific gene. It is possible to distinguish two categories of monogenic diabetes: diabetes caused by a defect of β cells function and diabetes caused by defect of insulin action. MODY diabetes (Maturity-Onset Diabetes of the Young) belongs to the first group. It is characterized by an early hyperglycemia at a young age, usually before the 25 years, by a lack insulin secretion and it has an autosomal dominant transmission. There have been identified different type of MODY, each caused by a single mutation in a specific gene: MODY 1 caused by mutation in HNF-4a gene, MODY 2 determined by GCK gene, MODY 3 caused by HNF-1a gene, MODY 4 due to the mutations in IFP-1 gene, MODY 5 determined by mutations in HNF-10 gene, MODY 6 caused by NeuroD1/BETA2 gene, MODY 7 associated to the mutations in KLF11 gene, MODY 8 associated to the mutations in CEL gene, MODY 9 caused by mutations in PAX4 gene, MODY 10 caused by mutations of the insulin gene, MODY 11 determined by mutations in BLK gene. There is another type of MODY, defined MOD-Y, that include all MODY cases, due to mutations in different genes than those typical of the classical types of MODY (1).

MODY 2 monogenic diabetes usually appears under 18 years old, with a frequency of about 5, 0-4-0, 1%, but it is always underestimated because it is often asymptomatic (the earliest hyperglycemia is typically between 5, 5 mmol/L and 8, 0 mmol/L) or it is diagnosed during routine screening or in the course of gestational diabetes. MODY2 clinical presentation is different from type 2 diabetes: MODY2 is absent weight gain, and it is an autosomal dominant transmission. There have been identified different type of MODY, each caused by a single mutation in a specific gene: MODY 1 caused by mutation in HNF-4a gene, MODY 2 determined by GCK gene, MODY 3 caused by HNF-1a gene, MODY 4 due to the mutations in IFP-1 gene, MODY 5 determined by mutations in HNF-10 gene, MODY 6 caused by NeuroD1/BETA2 gene, MODY 7 associated to the mutations in KLF11 gene, MODY 8 associated to the mutations in CEL gene, MODY 9 caused by mutations in PAX4 gene, MODY 10 caused by mutations of the insulin gene, MODY 11 determined by mutations in BLK gene. There is another type of MODY, defined MOD-Y, that include all MODY cases, due to mutations in different genes than those typical of the classical types of MODY (1).

Materials and methods

In the research were studied 33 index cases and, later, following positive emerged from the screening, were recruited 13 secondary cases, which showed some degree of kinship with the index cases. The subjects belonging to the group of index cases have come under our observation, at the Unit of Medical Genetics of the Hospital “Bianchi-Melacarino-Morelli” of Reggio Calabria, during the period between November 2009 and October 2011. This patients were sent from the center of diabetology of the city area and from that center, distributed in the region area, belonging to the Pediatric Diabetes Network of Calabria, established at regional level with 14 different centers. Patients were selected for the study of DNA by specific inclusion criteria: early age onset of diabetes (under 25 years old), mild hyperglycemia (100-130 mg/dL fasting) present at birth, absence of markers (auto-antibodies) indicative of type 1 diabetes, absence of obesity and family history of diabetes with at least two consecutive generations affected (10, 11). As for pregnant women, were selected those that had a familiar. The index cases have an age between 3 and 43 years, including 21 females and 12 males and inside them have been identified two categories of studies: patients with onset below 25 years (25 cases) and in pregnant subjects (8 cases). The secondary cases have an age between 8 and 72 years and they include 1 male and 12 females. Among them, 10 individuals are secondary cases related to the age group 3-21, while 3 are referred to the pregnant women group. In total 46 individuals were studied, 13 males and 33 females, with an age between 3 and 72 years. GCK gene mutational analysis has been realized by the technique of the DNA automated sequencing, whose basic steps are: DNA extraction from whole blood (through the use of the kit Qiagen DNA of the company Qiagen), PCR (under the following conditions: denaturation at 95°C for 30 seconds; annealing at 58°C for 1 minute and 30 seconds; extension at 72°C for 1 minute and 30 seconds. The primers used for the amplification reaction and the next sequencing reaction are those already reported by Sacchetti and coll. of Naples University “Federico II” (12) and listed in table 1 and subsequent verification on agarose gel, PCR purification, pre-sequencing (using the Qiagen purification kit), sequencing reaction (using the BigDye 3.1 cycle sequencing kit of Applied Biosystem with the following conditions: denaturation at 96°C for 20 seconds; annealing at 50°C for 5 second; extension at 60°C for 4 minutes), sequencing purification, post-sequencing (using the Qiagen Dye-ex purification kit), capillary electrophoresis on the automatic sequencer to eight capillary Applied Biosystem 3500
Genetic Analyzer. Each sequence was analyzed with the proprietary software of the Genetic Analyzer. Each sequence was analyzed using the_nucleotide sequence alignment software Polyphen-2 of the Harvard University.

### Results

#### Mutations and polymorphisms identified in the study patients

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Description</th>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS8+1G&gt;A</td>
<td>Intronic mutation</td>
<td>Nonsense</td>
<td>Kjær et al., 2007</td>
</tr>
<tr>
<td>IVS1+1G&gt;T</td>
<td>Intronic mutation</td>
<td>Nonsense</td>
<td>Kjær et al., 2007</td>
</tr>
<tr>
<td>W167X</td>
<td>Missense mutation</td>
<td>Nonsense</td>
<td>Kjær et al., 2007</td>
</tr>
</tbody>
</table>

The mutation IVS8+1G>A determines the substitution of the first G of the ATG triplet in a C, to obtain the triplet ACG. The consequence of this nucleotide change is manifested at the amino acid level, where the replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG.

The mutation was identified in two cases. It determines the substitution of the first G of the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG. The replacement of the T belonging to the ATG triplet in a C, to obtain the triplet ACG.
heterozygous, in position -30 bp from 5'-UTR. This polymorphism has been already reported in the literature in 2005 by Rose et al. and it is very significant in determining the hyperglycemia (17). It plays a special role in facilitating the bond with the transcription factors, which are important for the transcription process that leads to the mRNA formation, which is necessary for the subsequent glucokinase protein synthesis. Therefore, it is essential in the protein regulation because, in the presence of this change, the binding of transcription factors with the promoter is a disadvantage, by determining the decrease of gene expression. At phenotypic level, this situation means an increase of hyperglycemia. Based on these bibliographic information and followed the results obtained from the analysis it was possible to define the subject negative at diagnosis of MODY 2 diabetes. However, it is important to keep in mind the presence of this polymorphism because it is closely linked to the blood glucose altered values, presented in the studied subject. This polymorphism is not only in association with hyperglycemia, but encourages the development of CAD: Coronary Artery Disease and, among patients with CAD, it increases the development of type 2 diabetes (20; 21). The change in the promoter region identified in this study does not show a close link with MODY 2 diabetes, but it still explains the values of hyperglycemia and it represents a susceptibility for particular future clinical conditions but, more importantly, has special significance with regard its role at the molecular level.

The nucleotide exchange IVS6+8T>C belongs to polymorphism group, which, during, were identified with high frequency both in the positive subjects both in the negative ones. In fact, in the 46 studied index cases, 31 were negative. Among these negative, 13 cases presented the IVS6+8T>C polymorphism on exon-intron junction of the 9 exon at the polymorphism position -8. Data reported in the literature show the frequency with which one can find the polymorphism, based on the percentage with which the polymorphism is found in the population.

References