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Research paper

Maturity Onset Diabetes of the Young (MODY) in Tunisia: Low frequencies of *GCK* and *HNF1A* mutations

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ABSTRACT

Maturity Onset Diabetes of the Young (MODY) is a monogenic form of diabetes characterized by autosomal dominant inheritance, an early clinical onset and a primary defect in β-cell function. Mutations in the *GCK* and *HNF1A* genes are the most common cause of MODY among Caucasians. The etiology of MODY in Tunisia stills a challenge for researchers. The aim of this study was to screen for mutations in *GCK*, *HNF1A*, *HNF4A* and *INS* genes in North African Tunisians subjects, in whom the clinical profile was very suggestive of MODY. A total of 23 unrelated patients, with clinical presentation of MODY were tested for mutations in *GCK*, *HNF1A*, *HNF4A* and *INS* genes, using Denaturing High Performance Liquid Chromatography (DHPLC), Multiplex Ligation-depend Probe Amplification (MLPA) and sequencing analysis. We identified the previously reported mutation c-169C > T in one patient as well as a new mutation c-457C > T in two unrelated patients. No mutations were detected in the *HNF1A* and *INS* genes. Despite restrictive clinical criteria used for selecting patients in this study, the most common genes known for MODY do not explain the majority of cases in Tunisians. This suggests that there are others candidate or unidentified genes contributing to the etiology of MODY in Tunisians families.

1. Introduction

Maturity Onset Diabetes of the Young (MODY) is a genetically heterogeneous form of monogenic diabetes, characterized by an early onset (usually before 25 years of age), autosomal mode of inheritance and a primary defect in pancreatic β-cell function (Owen and Hattersley, 2001; Fajans et al., 2001). According to the Online Mendelian Inheritance in Man database (MIM # 606391), pathologic mutations in thirteen genes have been described in various MODY subtypes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *TCF2*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8* and *KCNJ11*) (http://omim.org/). However, most studies have shown that HNF1A-MODY and GCK-MODY are the most prevalent forms (Velho and Robert, 2002). Their relative frequencies vary considerably among ethnics group. While HNF1A-MODY mutations are the most common cause of suspected MODY patients in the United Kingdom (53%) (Shields et al., 2010) and Germany (62%) (Shober et al., 2009), GCK-MODY are the most common cause of suspected MODY subjects in Spain (80%) (Estalella et al., 2007), France (56%) (Froguel et al., 1993), and Italy (41%) (Froguel et al., 1993). The other forms of MODY are less common among studied populations (Shields et al., 2010). Besides individual population characteristics, recruitment criteria account for part of the difference between these two most common types. Children from general population screened regardless of glyceamic status show higher rate of GCK-MODY, whereas MODY typing of adult diabetic patients yields a higher prevalence of HNF1A-MODY (Estalella et al., 2007; Giuffrida and Reis, 2005). Their remains a group of families with autosomal dominant segregation of non-autoimmunity diabetes mellitus for whom the genetic culprit is still to be identified and is known as MODY X. It accounts for approximately 15–20% of Caucasians families fitting MODY criteria (Kim, 2015; Mantovani et al., 2003).

In Tunisia, a previous study showed the absence of mutations in known MODY genes in 11 patients (Amara et al., 2012). The aim of the present study was to investigate the contribution of the MODY genes *HNF4A*, *GCK*, *HNF1A* and *INS* in the etiology of 23 unrelated Tunisian families.

Abbreviations: Apo A, apolipoprotein A; Apo B, apolipoprotein B; BMI, body mass index; DHPLC, Denaturing High Performance Liquid Chromatography; GAD, Anti-Glutamic acid Decarboxylase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HLA-DRB1, HLA class II histocompatibility antigen, DRB1 beta chain; hs-CRP, high sensitive C reactive protein; IA2, Anti-tyrosine phosphatase; Lp(a), lipoprotein a; MLPA, Multiplex Ligation-depend Probe Amplification; MODY, Maturity Onset Diabetes of the Young; PCR, polymerase chain reaction; SPSS, statistical Package for Social Sciences; SSO, enzyme-Specific Oligonucleotid

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2. Patients and methods

2.1. Patients

We analyzed 23 extended families. They were recruited from the Unit of Endocrinology of Monastir Hospital as well as several clinical centers located in the Sahel region of Tunisia. The main criteria used for the selection of these unrelated families were autosomal dominant mode of inheritance of diabetes mellitus, presence of the disease in at least three consecutive generations, presence of at least one proband with early onset diabetes initially not requiring insulin treatment, and normal Body Mass Index (BMI). In addition, 50 unrelated, healthy subjects served for the control of the DNA sequences. For blood collect, we have obtained approval from the Hospital authority and the ethics committee (No: 13/2010) after consent of patients.

2.2. Clinical data

Details of patients such as age, duration of diabetes, BMI, complications and current treatment were recorded. Blood samples were drawn for measurement of fasting glucose, HbA1c, insulin, C-peptide, creatinine, uric acid, cystatin C, high sensitive C reactive protein (hs-CRP), lipid profile (triglycerides, total cholesterol, HDL-cholesterol (HDL-C)), Apolipoprotein A and B (Apo A and Apo B), Lipoprotein a (Lp (a)), Anti-Glutamic acid Decarboxylase (GAD) and Anti-tyrosine phosphatase (IA2).

2.3. Assays

Fasting glucose, Urea, Creatinine, Uric acid, Triglycerides, Total Cholesterol and HDL-C were evaluated on Beckman auto-analyzer by using commercial kits from Randox (Randox Diagnostics, Antrim, UK). HbA1c, Apo A, Apo B, Lp (a), cystatin C and hs-CRP were determined by immunoturbidimetric method (Roche Diagnostics, Mannheim, Germany). Fasting insulin, C-peptide were determined by chemiluminiscent microparticule immunoassay (Abbott Diagnostics, Rungis, France). Anti- GADA and Anti IA2 were performed by radio-immunoassay technology for all MODY patients.

2.4. Mutation screening

Genomic DNA was extracted from peripheral blood lymphocytes using phenol-chloroform method.

All *GCK* (MIM #138079) exons, including intron/exon boundaries and the promoter, were screened by PCR-DHPLC. In order to generate heteroduplices, amplified fragment were denaturated at 95 °C for 5 min, then slowly cooling. Each fragment was analyzed using WAVE DNA Fragment Analysis system (Transgenomic, Omaha, NE). A lineal gradient of buffer A (containing (0.1 mol/L triethylammonium acetate–TEAA) and buffer B (0.1 mol/L TEAA, 25% acetonitrile) was used to elute DNA from the column. Primers, PCR and DHPLC conditions are available on request. The exons of those patients with aberrant wave profile were subjected to sequence analysis on an ABI 3130 capillary DNA Analyzer (Applied Biosystems).

Exons, flanking introns and promoters of the *HNF1A* (MIM #142410), *HNF4A* (MIM #600281) and *INS* (MIM #176730) genes were amplified by PCR and then screened for mutations in probands by direct sequencing. Primers and PCR conditions are available upon request.

Families without a mutation in the four analyzed genes detectable by the previously described methods were screened with by Multiplex ligation-dependent probe amplification (MLPA), following the manufacturer's instructions (MLPA; MRC-Holland, Amsterdam, Holland). Commercially oligonucleotides probes for *GCK*, *HNF1A* and *HNF4A* exons were used.

When a mutation is identified in a proband, all the available family members were analyzed for the appropriate exon in order to perform the corresponding segregation analysis.

The Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk), the genome browser Ensembl (www.ensembl.org) and the previously published MODY mutation detection articles (Ellard and Colclough, 2006; Osbak et al., 2009) were used to ascertain whether the variant was novel or not.

2.5. HLA-DRB1 typing

HLA-DRB1 genotyping was performed by the Polymerase Chain Reaction enzyme-Specific Oligonucleotid method (PCR-SSO) combined with Luminex technology as described (Urrutia et al., 2017).

2.6. Statistics

Statistical analysis was performed using statistical Package for Social Sciences (SPSS for windows, version 19.0). Results were expressed as means ± standard deviation (M ± SD). For quantitative traits, student's *t*-test was used. $P < 0.05$ was considered statistically significant.

3. Results

Anti- GADA and Anti IA2 were absent in all subjects. Three index cases (13.05%) were shown to carry mutations in *GCK* and *HNF4A* genes. No molecular defect was revealed by the MLPA method. A rate of 86.95% could not be genetically explained in our study.

We identified a previously described mutation c.-169C > T in the *HNF4A* gene in one family. A novel mutation, c.-457C > T, was also identified in two unrelated subjects. Segregation of this mutation in the two families wasn't possible as members refused to adhere to our study.

3.1. Patient with the HNF4A mutation c.-169C ≥ T

By analysising the *HNF4A* gene, we identified the c.-169C > T mutation in a proband as well as her mother (Fig. 1a). This mutation is located in the promoter region P2 (exon 1d).

The HNF4A-MODY patient was a 15 years-old nonobese (BMI = 19.47 Kg/m^2) female, from consanguineous parents. She was at term with a birth weight of 3.9 Kg. The neurological and physical developments were normal. The patient was diagnosed on the time of the study. The blood glucose, the Hb1Ac and the C-peptide levels were respectively 8.89 mmol/L, 9.16% (12 mmol/L) and 0.53 ng/mL. Physical examination revealed no other underlying disease. The patient was treated with insulin. Her family history was strongly positive for diabetes. The patient's sister, her mother and her grandfather on her mother's side were diagnosed with diabetes (Fig. 1b). Her mother was diagnosed with diabetes after presenting polyuria-polydipsia and nocturia syndromes. She was treated with OHA than with insulin injection. Her father wasn't diabetic, but he presented hypertension at 41 year-old.

3.2. Patient with GCK promoter variation c.–457C ≥ T

The analysis of the *GCK* gene revealed a novel variation c.-457C > T in two unrelated probands (Fig. 2a). This substitution of cytosine to tyrosine, located in the promoter region P1, was absent in 50 non-diabetic controls.

The first patient was a nonobese (22.37 Kg/m^2) female of 24 year-old. She was from unrelated parents. She was born at term with a birth weight of 3.150 Kg. Her physical and neurological development were normal. The patient was diagnosed at 19 year-old, during the first pregnancy when routine testing was performed. Blood glucose, HbA1c and C-peptide levels were respectively 7.62 mmol/L, 7.3% (9 mmol/L) and 0.87 ng/mL. Physical examination revealed no other underlying