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Screening of mutations in the *GCK* gene in Jordanian maturity-onset diabetes of the young type 2 (MODY2) patients

R. Khalil[1], F. Al-Sheyab[2], E. Khamaiseh[2], M.A. Halaweh[1] and H.A. Abder-Rahman[3]

[1]Biotechnology and Genetic Engineering Department, Philadelphia University, Jordan
[2]Genetic Engineering and Biotechnology Department, Jordan University of Science and Technology, Jordan
[3]Forensic Medicine and Pathology Department, Faculty of Medicine, The University of Jordan, Jordan

Corresponding author: R. Khalil
E-mail: r_khalil@philadelphia.edu.jo

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ABSTRACT. Maturity-onset diabetes of the young type 2 (MODY2) is a genetic form of diabetes mellitus caused by mutations in the glucokinase gene (*GCK*). We assessed the frequency of *GCK* gene mutations in Jordanian suspected MODY2 patients. We screened exons 7, 8 and 9, which are specific for pancreatic glucokinase, for mutations at positions 682A>G, p.T228A; 895G>C, p.G299R, and 1148C>A, p.S383X, respectively, in 250 subjects (100 patients suspected to have MODY2 and 150 healthy controls without family history of diabetes mellitus). We did not find any association of these mutations in Jordanian suspected MODY2 patients or in healthy controls, different from data on Caucasian Italian patients screened for the same mutations.

Key words: Glucokinase; MODY2; Mutation; Jordanian

INTRODUCTION

Glucokinase is a member of the hexokinase family and plays a key role in glucose homeostasis as a glucose sensor in pancreatic β-cells. It catalyses the initial step in these pathways, ATP (adenosine triphosphate)-dependent phosphorylation to form glucose-6-phosphate (G-6-P) (Matschinsky et al., 1993). A reduction in β-cell glucokinase (GCK) amount or activity increases the glucose threshold for insulin secretion, causing typical fasting hyperglycemia (Byrne et al., 1994). GCK is expressed in pancreatic β-cells, hepatocytes and a variety of neural/neuroendocrine cells, including pancreatic α-cells, L- and K-gut enterocytes and selected neurons (Schuit et al., 2001). Although GCK from pancreas, liver and brain are similar in kinetic activity and are coded by the same gene with 12 exons on chromosome 7 (7p15.3 - p15.1), their primary structures at the N-terminal end are different due to distinct splicing of the RNA transcript. The GCK enzyme contains 465 amino acids and exon 1 varies in diverse tissues due to the different promoter regions: the upstream promoter is functional in pancreas and brain, the downstream promoter is used only in liver (Stoffel et al., 1992; Gloyn, 2003).

In view of its crucial role in the regulation of glucose-stimulated insulin secretion, it is possible that mutations in the *GCK* gene can cause both hyperglycemia and hypoglycemia. Genetic studies have shown that *GCK* mutations are responsible for maturity-onset diabetes of the young type 2 (MODY2). MODY2 is a genetically and clinically heterogeneous form of diabetes mellitus, characterized by an early age at onset, a primary defect in β-cell function and an autosomal dominant inheritance (Fajans et al., 2001). Among the different types of MODY diabetes described thus far, each is due to a different gene mutation (HNF4A, GCK, HNF1A, IPF1, HNF1B, NEUROD1, CEL) (Raeder et al., 2006; Weedon and Frayling, 2007). The GCK MODY form is caused by mutations in the *GCK* gene. Thus far, about 200 *GCK* mutations have been reported and their frequency is higher in European Caucasians, particularly in those from France and Italy (Pinterova et al., 2007).

Missense mutations of *GCK* represent the most frequent cause of MODY2; to date more than 200 mutations with distinct enzymatic characteristics have been found. These mutations were also detected in 5-6% women with gestational diabetes (Ellard et al., 2000). Heterozygous activating missense *GCK* mutations causing persistent hyperinsulinemic hypoglycemia of infancy and inactivating homozygous *GCK* mutations leading to permanent neonatal diabetes mellitus are much less frequent (Gloyn, 2003).

Since there is no previous study focusing on the screening of the *GCK* gene mutations in Jordan, this study was the first and established grounds for a preliminary screening aimed to assess the presence of the *GCK* gene mutations in Jordanian MODY2 patients at the positions 682A>G, p.T228A; 895G>C, p.G299R, and 1148C>A, p.S383X and compared the results with a similar study previously conducted in a Caucasian Italian MODY2 population.

MATERIAL AND METHODS

Subjects

One hundred patients were recruited from the National Center for Diabetes, Endocrine and Genetic Diseases, King Hussein Clinical Hospital, University of Jordan Hospital and Islamic Hospital in Amman, King Abdullah University Hospital, Yarmok University Clinical Center, and Prince Basmah Hospital in Irbid, all in Jordan.

The patients aged 18-27 years, 57 males and 43 females, whose clinical presentation was suggestive of MODY2 were selected for *GCK* gene mutation detection. Inclusion criteria were: early onset (by 25 years) of diabetes, mild hyperglycemia, no autoimmune markers of type I diabetes, without obesity and positive family history of diabetes for at least two consecutive generations for most of the patients. No treatment was administered to the patients and no diabetes complications were evident up to diagnosis. A fasting blood sample was drawn from patients and from 100 unrelated controls, who were from the same geographical area, and used for the GCK molecular characterization. Informed consent, in accordance with guidelines of the above-mentioned centers and hospitals, was obtained from each patient or normal control subject.

Clinical and biochemical examination

We determined the clinical and biochemical parameters for each patient subject upon diagnosis (Table 1): age, age at diagnosis, fasting blood sugar, weight, height, family history of diabetes, body mass index, and glycosylated hemoglobin. The same parameters were determined for the control subjects.

Table 1. Clinical and biochemical characteristics of MODY2 patients and control subjects.

Parameter	Patients (N = 100)	Controls (N = 150)
BMI (kg/m^2)	30.87 ± 2.5	24.1 ± 0.4
Age (years)	25.4 ± 0.5	21.6 ± 1.5
Height (cm)	168.2 ± 3.5	168.2 ± 2.7
Weight (kg)	76.5 ± 5.3	67.7 ± 6.1
Males	57 (57%)	80 (53.3%)
Females	43 (43%)	70 (46.7%)
FBS (mg/dL)	178.5 ± 5.8	110 ± 5.1
HbA1c (%)	10.44 ± 0.5	5.07 ± 0.58
Age at diagnosis (years)	30.0 ± 2.0	-
Positive family history	70 (70%)	50 (33.3%)
Negative family history	30 (30%)	100 (66.7%)

BMI = body mass index; FBS = fasting blood sugar; HbA1c = glycosylated hemoglobin. Values are reported as means ± SD or as number of subjects with percent in parentheses.

DNA extraction

Genomic DNA from patients and controls was extracted from whole blood plus EDTA using Wizard Genomic DNA Purification kit according to manufacturer recommendations (Promega, USA).

Polymerase chain reaction analysis

Detection of the mutations of exons 7, 8, and 9 of *GCK* gene was performed by polymerase chain reaction (PCR), using previously reported primers (Matschinsky, 2002). The PCR mixture contained in a final volume of 25 µL: 20 mM of each primer, 1X PCR buffer (Promega), 10 mM of each deoxynucleotide triphosphate (Promega, UK), 2.5 units Taq DNA polymerase (Applied Biosystems) and 200 ng genomic DNA. Each PCR was performed in a thermocycler (BioRad, Italy, MJ-BioRad, Italy).

The amplification conditions for each exon consisted of two initial cycles at 94°C for 30 s, 52°C for 1 min, and 72°C for 1 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by 5 min at 72°C, and ending at 4°C.

The PCR products were evaluated by agarose gel electrophoresis at 100 V for 30 min.

The primer list and expected size of PCR amplification products are shown in Table 2.

Table 2. Primers used for amplification of exons 7, 8 and 9 of *GCK* gene and the expected length of PCR products.

Nucleotide changes	Exon	Amino acid changes	Restriction enzyme
682A>G	7	Thr228Ala	*Bst*UI
772G>T	8	Gly299Arg	*Hha*I
1148C>A	9	Ser383Tyr	*Bfa*I

Oligonucleotides were synthesized by Alpha DNA, Canada.

Restriction fragment length polymorphism

To screen the missense mutations at positions T228A and G299R and a nonsense mutation S383X in exons 7, 8, and 9, respectively, the amplified PCR products for exons 7, 8, and 9 were cleaved using *Bst*UI, *Hha*I and *Bfa*I restriction endonucleases, respectively. The PCR products were treated with 5 units of restriction endonuclease. The mixture was incubated at 37°C for 24 h, and then electrophoresed on 2-3% agarose gels at 100 V for 30 min. The positive controls for the mutations were gifts from Dr. Vilma Mantovani (Centro Ricerca Biomedica Applicata, Bologna, Italy).

The description of restriction recognition sites, the lengths of the PCR products, restriction products, and screened mutant amino changes by each endonuclease are shown in Table 3 and Table 4, respectively.

Table 3. The screened *GCK* gene mutations and restriction enzymes.

GCK gene		Primer sequence	Size of PCR product (bp)
Exon7	F	5'- TGCAGCTCTCGCTGACAGTCC -3'	287
Exon7	R	5'- CTCCCATCTGCCGCTGCACC -3'	
Exon8	F	5'- CGTGCCTGCTGATGTAATGG -3'	268
Exon8	R	5'- GCCCTGAGACCAAGTCTGC -3'	
Exon9	F	5'- CTGTCGGAGCGACACTCAG - -3'	410
Exon9	R	5'- CCCCCAAATCTAGGCCAAGG -3'	

Table 4. The restriction enzymes, their recognition sites and the expected RFLPs of the *GCK* genes.

GCK gene	Restriction enzyme	Recognition site	RFLP resultant fragment (bp)	
			Normal	Mutant
Exon 7	*Bst*UI	5' …CG▼CG… 3'	(287)	245
		3'…..GC▲GC… 5'	No cutting	42
Exon 8	*Hha*I	5' …GCG▼C… 3'	(268)	No cutting
		3'…..C▲GCG… 5'	116 bp	
			152 bp	
Exon 9	*Bfa*I	5' …C ▼TAG… 3'	(410)	323
		3'….. GAT ▼C … 5'	398	75
			12	12

RESULTS

The PCR products were screened for the presence of Thr228Ala missense mutation in exon 7 of the *GCK* gene where the A changes to G at nucleotide 682 for the restriction transition profile for the *Bst*UI restriction enzyme. The wild-type allele is resistant to digestion by *Bst*UI while the mutant allele produces fragments of 245 and 42 bp.

An *Hha*I restriction enzyme is used to genotype the Gly299Arg missense mutation in exon 8 of the *GCK* gene in which G changes to T at nucleotide 772. An *Hha*I restriction digestion in the wild-type allele yields two fragments of 116 and 152 bp, while the mutant allele lacks the *Hha*I recognition site.

The detection of the Ser383Tyr nonsense mutation at nucleotide 1148, where C changes to A was by *Bfa*I restriction enzyme. A *Bfa*I digestion of the mutant allele yields three fragments of 323, 75, and 12 bp, while the wild-type allele yields two fragments of 398 and 12 bp.

The screening of the *GCK* gene mutations among the Jordanian MODY2 suspect and healthy control subjects using PCR-restriction fragment length polymorphism analysis showed no observed mutants in both MODY2 and healthy control subjects. The PCR products for all the amplified products followed the same pattern of restriction action in both patients and controls. Therefore, no observed Thr228Ala, Gly299Arg and Ser383Tyr mutations were detected in the MODY2 diabetics or normal control subjects. Thus, the results are different from a previous study that detected the presence of the above-mentioned mutations in Caucasian Italians from the Oxford region in UK MODY2 patients.

DISCUSSION

MODY is a relatively rare form of diabetes mellitus, and some studies suggest that it may not be so uncommon as hypothesized and that 2-5% of patients with diabetes mellitus type 2 may in fact have MODY (Ledermann, 1995).

Recent data support the finding that MODY is prevalent in approximately 1-2% of diabetic patients in Europe (Owen and Hattersley, 2001). MODY2 and MODY3 represent the most common forms of MODY in Europe. The relative prevalence of MODY2 among all MODY patients varies greatly in studies from different populations: from 46-56% in France (Froguel et al., 1993; Velho et al., 1997), 41-61% in Italy (Massa et al., 2001; Mantovani et al., 2003), 25-41% in Spain (Costa et al., 2000; Barrio et al., 2002), and 31% in the Czech Republic (Pruhova et al., 2003) to 11-20% in the UK (Thomson et al., 2003), 10% in Denmark (Johansen et al., 2005), 8% in Germany (Lindner et al., 1999), and 3.5% in Scandinavia (Lehto et al., 1999). The clinical features of MODY2 are usually mild, and diagnosis is often accidental (Hattersley, 2005). MODY2 is caused by mutations in the *GCK* gene.

The identification of a *GCK* mutation in subjects whose clinical phenotype is suggestive of MODY usually distinguishes patients with a benign prognosis (GCK MODY) from those with severe hyperglycemia (other MODY forms) because the diagnosis cannot always be made on clinical grounds alone. Moreover, according to the variations in the prevalence risk of MODY2 among different populations, we decided to screen *GCK* gene mutations in Jordanian MODY2 populations and to determine racial differences. Furthermore, a recent study revealed an increase in the prevalence of diabetes mellitus in Jordan over 10 years (Ajlouni et al., 2008).

In this study, we did not find positive cases for the *GCK* gene mutation at positions 682A>G, p.T228A, 895G>C, p.G299R and 1148C>A, p.S383X, respectively, in 100 patients with suspect MODY2 in comparison to the positive control DNA samples for those mutations in Italian MODY2 patients, so there was no difference between MODY2 and healthy control subjects.

However, previous studies found that the *GCK* gene mutations occur in 6% of cases in Italian and Norwegian populations, and between 8 and 56% in southern European countries (Shehadeh et al., 2005; Stern et al., 2007; Sagen et al., 2008).

Thus, this preliminary study cannot exclude the possibility that the *GCK* gene is a risk gene in the pathogenesis of MODY2 in the Jordanian populations. Therefore, wide spectrum investigations of *GCK* gene mutations in MODY2 Jordanian populations and variants are strongly recommended and worthwhile.

In conclusion, molecular screening is useful in the diagnosis of MODY because it allows the physician to confirm the diagnosis and to predict the severity of the mutation and prognosis as well as the clinical course of the patient.

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