



Molecular detection of Glucokinase (GCK) Gene Mutation in Maturity-onset diabetes of the young (MODY2) among Sudanese families With Type 2 Diabetes Mellitus

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ABSTRACT

Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of non-insulin dependent diabetes mellitus (NIDDM), it's a heterogeneous group of disorders characterized by dysfunction of beta-cells, and usually referred to monogenic forms of diabetes mellitus to distinguish them from the common types of disease such as type 1 or type 2 diabetes (M. Vaxillaire et al. 2008). Aim: The aim of this study was to examine the prevalence and nature of mutations in GCK gene among Sudanese families.

Exons 7 were screened, which are specific for pancreatic glucokinase, mutations at positions 682A>G, p.T228A; in 160 subjects (80 patients suspected to have MODY2 and 80 healthy controls without family history of diabetes mellitus). By using polymerase chain reaction (PCR) the target part of GCK gene was amplified, the amplified regions were digested by using restriction enzymes: BstUI and electrophoresed on agarose gel. Results of mutation were screened using restriction fragment length polymorphism and confirm by using DNA sequencing.

The results showed that, there was no any association of these mutations in Sudanese suspected MODY2 patients or in healthy controls. On the other hand during analysis of the DNA sequences observed three synonymous mutations, in one family that may have significant effect on the protein function

In conclusion, the study indicates that mutations in GCK/MODY2 at position 682A>G, p.T228A is not detected in Sudanese families

KEYWORDS: Glucokinase; MODY2; Mutation; Sudanese family

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المستخلص

الغلوكوكينيز هو عبارة عن أنزيم يلعب دور مهم في الاستجابة للغلوكوز بواسطة خلايا بيتا في البنكرياس و يدخل في أيض الغلوكوز في خلايا الكبد وأي خلل في هذا الأنزيم يؤدي إلي خلل في إفراز الأنسولين في البنكرياس وفي إنتاج الغلوكوز في الكبد وكليهما يؤديان إلي مرض السكري.

وقد تم اخذ 80 عينة دم من 9 عوائل للابن لاهم تاريخ مرضى للسكر الوراثي و80 شخص من الاصحاء من الذين ليس لديهم تاريخ مرضى للسكر في العائلة لمسح جين الغلوكوكينيز للطفرة الوراثية (G>A82, p.T228A) في الاكسون 7 باستخدام نظام التحليل (PCR-RFLP) ولم نجد هذه الطفرة في ال 80 عينة لمرضى السكر او الاشخاص الاصحاء وقد تعزى هذه النتيجة السلبية لقلّة عدد العينات المستخدمة في التحليل. وبارسال بعض العينات لتحليل ال (DNA sequences) وجدنا بعض الطفرات (ynonymous mutations) في احدى العوائل التي قد تكون لها علاقة بتغيير البروتين التي قد تسبب مرض السكري

INTRODUCTION

Glucokinase is a member of the hexokinase family. (Ellard S, et al. 2009) and plays a key role in glucose homeostasis as a glucose sensor in pancreatic β -cells.(Matschinsky FM, 1968; Matschinsky FM. et al. 2009) To date more than 200 missense mutations of GCK with distinct enzymatic characteristics have been found which represent the most frequent cause of MODY2. (Ellard S, et al. 2008) Glucokinase is located on the chromosome 7 (7p15.3-15.1) (Tinto N, et al. 2008) and contains 12 exons that encode the 465-amino-acid protein Glucokinase.(National center for biotechnology) Heterozygous inactivating mutations of the GCK gene lead to mild fasting hyperglycemia and diminished insulin secretion, along with decreased hepatic glucose uptake and glycogen synthesis (NoorianS, et al. 2013) Over 99% of MODY with a known genetic etiology results from mutations in hepatocyte nuclear *HNF1- α* (formerly MODY3), glucokinase (*GCK*) (MODY2), or *HNF4- α* (MODY1). (Shields B, et al. 2010) While mutations in other genes have been shown to cause MODY, (Murphy R, et al.2008) are very rare and genetic testing is not recommended unless other syndromic features are present

(Ellard S, et al. 2008). Since there is no previous study focusing on the screening of the GCK gene mutations in Sudan, this study was the first and established grounds for a preliminary screening aimed to assess the presence of the *GCK* gene mutations in Sudan MODY2 patients at the positions 682A>G, p.T228A.

MATERIALS and METHODS

Subject's Total number of 80 family members with strong history of early-onset type 2 diabetes distributed into nine Sudanese families and another eighty control nondiabetic participants were enrolled in this study. Blood sample were collected from different area in Khartoum state and different tribe Robatab, Gaaleen, Kawahla, Nobioon (Mahas and Danagla), and others. Inclusion criteria were: early onset (by 25 years) of diabetes, mild hyperglycemia, without obesity and strong history family of diabetes for at least two consecutive generations for most of the patients

Biochemical investigations

Biochemical measurements were performed with a (COBAS INTEGRA 400 plus system, COBAS C 111, and cobas e 411)

for Glucose, HbA1c and C-peptide respectively. Table 4.2 shows the biochemical and anthropometric measurement of diabetic family member. Fasting blood glucose and HbA1c levels were highly significant among the family member than the controls (p. =0.000). The mean of Body mass index (BMI) of the family members was 26.1 ± 4 and control 24

± 4.0 showed significantly different between family members and control ($p = 0.004$).

C-peptide level showed highly significant between diabetic families (2.0 ± 0.7) and control (2.4 ± 0.6), (p. =0.004). Since sex, age and BMI are known to be independent risk factors for type2 diabetes.

Table 2. Biochemical anthropometric measurement of diabetic families and control subjects

Parameter	families members (N= 80) ¹ (mean \pm SD)	Controls (N = 80) (mean \pm SD)	p. value
Age (mean \pm SD)	35.6 \pm 16.9	31.4 \pm 13.1	0.078
Sex (F/M)	(47/33)	(42/38)	0.157
BMI (kg/ m ²) ²	26.1 \pm 4.0	24 \pm 4	0.004
Underweight < 20 (kg/ m ²)	7 (9%)	11 (13.7)	
Normal weight 20 – 25 (kg/ m ²)	25 (31%)	37 (46.3%)	
Overweight 26 – 30 (kg/ m ²)	31(39%)	28(35%)	
Obese > 30 (kg/ m ²)	17 (21%)	4 (5%)	
FBS (mg/dL) ³	196 \pm 84	99 \pm 14	0.000
HbA1c (%) ⁴	6.6 \pm 2	5.2 \pm 3	0.000
C-peptide (nmol/L) ⁵	2.0 \pm 0.70	2.4 \pm 0.6	0.000

¹Data is represented as mean \pm SD.

²BMI: Body Mass Index.

³ FBS: Fasting Blood Sugar; (80 – 126 mg/dL)

⁴The reference range (HbA1c =3.5 – 6.5%)

⁵The reference range of C-peptide is 0.8-3.1 ng/mL with type I < 0.8

Molecular analysis

Genetic testing for GCK was performed by the Department of Molecular Genetics, Institute Of Endemic Diseases Biomedical And Clinical Research in the University Of Khartoum.

DNA extraction

Genomic DNA from all patients and controls were extracted from whole blood using Genomic DNA Purification kit according to manufacturer recommendations (Blood

DNA Preparation Kit, Jena Bioscience, Germany)

Quantification of extracted DNA

Purity and concentration of extracted DNA were determined using Nanodrop ND 1000. The DNA sample was diluted with distilled water (10 + 90 μ l). Pure distilled water was used as a blank to calibrate the machine. The concentration of the DNA sample was then measured using 260/280 nm mode and measurement units μ g/ml. DNA purity was determined using purity scan mode.

Polymerase chain reaction (PCR):

The primers sequences are shown in table 1 were amplified by polymerase chain reaction (PCR). (PCR premix (iNtRON's, Korea). The PCR premix (iTaq) containing the following unit 2 unit Taq, dNTPs mixture (10Mm), 25mM MgCl₂ and 10x PCR buffer , 1μL of each primer and 2μl of

DNA, PCR was performed in a total volume of 25 μL using a thermocycler (SensoQuest, , Göttingen, Germany) Cycling condition for the PCR was as the following:95°C initial denaturation for 5 minutes, 35 cycles of: denaturation at 94°C for 30 sec, annealing at 61°Cfor 30 sec, 72°C elongation for 30 sec, 72°C final elongation for 10 min.

Table 2. The primer sequence and restriction enzymes of screened GCK gene

GCK gene	Primer sequence	Restriction enzyme
Exon7	F CCCTGAAAGCTGTCCCTGG	<i>Bst</i> UI
	R ACTTTGGGGAATGTTGGGTT	

Detection of PCR product

After PCR amplification, the PCR products were separated on a 1.5% Agarose gel stained with 3μl Ethidium Bromide in (1 X TBE buffer) which contain of (89 mMTris Base, 89 mM Boric Acid, 2 mM EDTA). 5μL from the PCR product was loaded on the gel and left to run in gel documentation system for 90 minutes at 80V, the amplified fragments were visualized by illumination with short wave ultraviolet light and photodocumented. Molecular weight of DNA bands were estimated in relation to standard 100bp DNA ladder

Analysis of the *Bst*UI Polymorphism in GCK Gene

The PCR products of exon 7 (Fig. 2) of GCK gene were screened for detection of Thr228Ala missense mutation at nucleotide 682 (A < G) depend on the restriction transition profile of the *Bst*UI restriction enzyme (Table 3). The *Bst*UI cut the mutant allele and yields two fragments (245 and 42 bp) while the wild-type allele is undigested. The PCR products were not cut and showed the same model of restriction action in both groups. So, no mutation of Thr228Ala was detected in the study groups

Restriction fragment length polymorphism

The PCR products were treated with 5 units of restriction endonuclease. The mixture which contain (0.2 μL Restriction enzyme, 5 μL PCR Product, 2 μL10X NEBuffer , 2.8 μL D.W,) was incubated at 37°C for 24 h, and then electrophoresed on 3% agarose gels at 100 V for 60 min. (Fig. 1) 1.5% agarose gel showing PCR products of exon 7 of GCK gene Lane 1: DNA ladder (100 bp). Other Lane PCR Products (685) bp. Table 4.2:

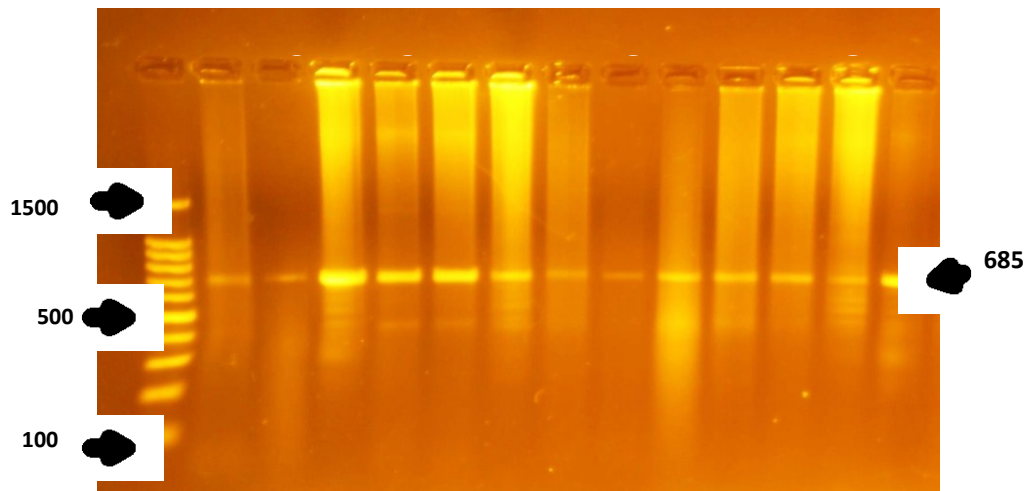
Bioinformatics Analysis: Sequencing of GCK Gene

DNA purification and standard sequencing was performed by Macrogen Company (Seoul, Korea). The sequences chromatogram was viewed by finchTV program. (<http://www.geospia.com/products/finchtv.s.html>).

Then the nucleotides sequences of the GCK- gene were searched for sequences similarity using nucleotide BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). (Atschul SF, et al. 1997) highly similar sequences were retrieved from NCBI and subjected to multiple sequence alignment using BioEdit software. (Hall TA .1999).

Table 3. The restriction site and RFLPs of GCK gene

Analyzed GCK	Restriction enzyme	PCR product Size (bp)	Restriction site	RFLP Fragment size(bp)	
				Normal	Mutant
Exon7	<i>Bst</i> UI	685	5' ...CG ▼ CG... 3' 3' ...GC ▲ GC... 5'	685 No cutting	490 195



(Fig. 1) 1.5% agarose gel showing PCR products of exon 7 of GCK gene Lane 1: DNA ladder (100 bp). Other Lane PCR Products (685) bp

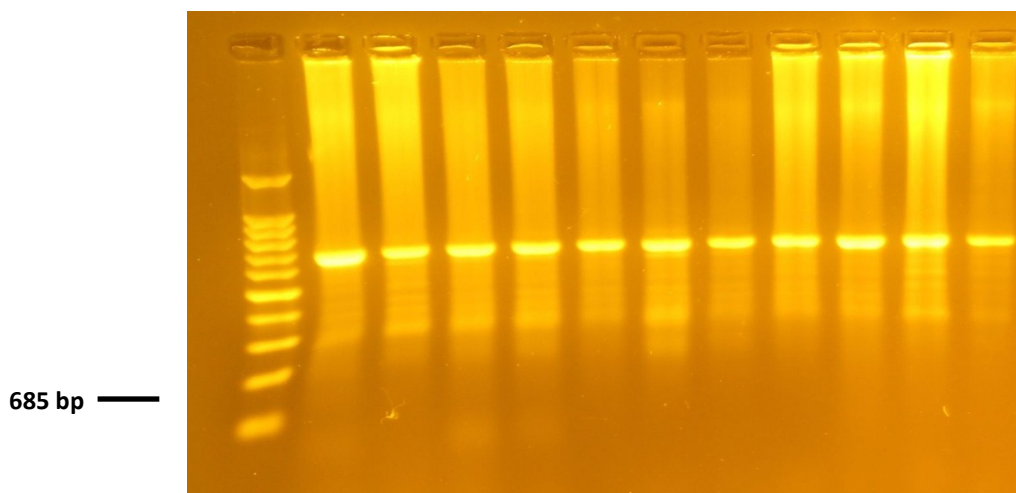


Fig. 2) 3% agarose gel showing restriction products of exon 7 of GCK gene by *Bst*UI Lane 1: 100 bp DNA ladder. Other Lane: Restriction PCR product

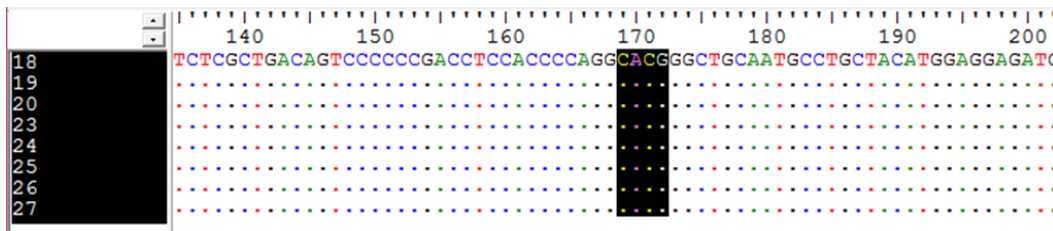


Fig. 3) Alignment determining by using BioEdit software. Highlight sequences indicated the position of BstUI cutting site in GCK Gene

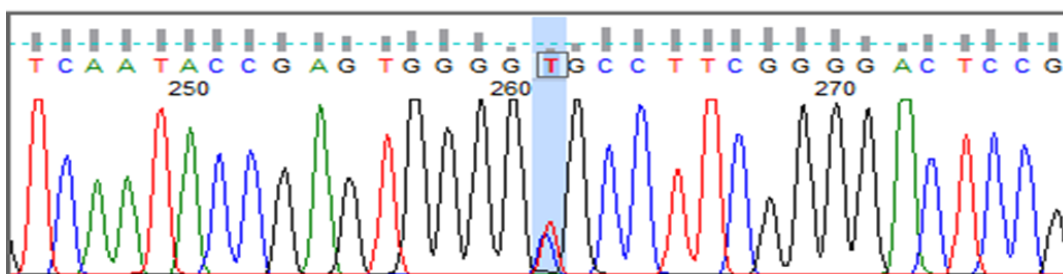


Fig. 4) the sequences chromatogram was viewed by FinchTV program which showed C > T substitution sequence

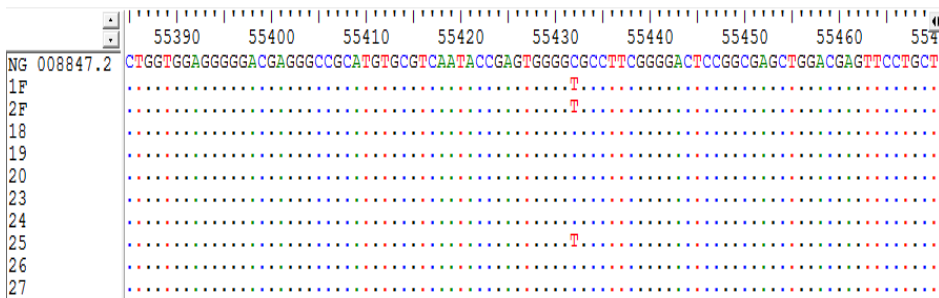


Fig. 5) Synonym mutation on three patients in one family

DISCUSSIONS

Glucokinase, which serves as a key regulating enzyme in insulin secretion stimulated by glucose, acts as the glucose sensor of pancreatic β -cells. To date, 620 mutations (missense, nonsense, frameshift, splice site, and promoter mutations and deletions) in 1441 families have been reported in the *GCK* gene, causing hypoglycemia and hyperglycemia. (Osbak KK, et al. 2009)

all participants had fasting blood samples taken for measurements of plasma glucose, glycosylated hemoglobin (HbA1C), and C-peptide our result showed that all families participate diabetic and non-diabetic in upper part of normal range different than control without history of diabetes also HAIC range (6.5 ± 3) on those with the clinical features of MODY and all results of C Peptide within normal

Some study show that patients with MODY2 usually have low rise in blood glucose, while their HbA1c is almost invariably below 7.5 % (Stride A, et al. 2002; Martin D, et al. 2008) similar to what is observed in this study. GCK gene missense mutation at positions 682A>G, p.T228A was searching in this study group by using Restriction fragment length polymorphism (RFLP), and confirm the result by sending sample for (DNA Sequences). Results obtained from the present investigation revealed that, no positive cases were detected.

These results agreed with Jordanian (library of university of Jordan. 2007) study which revealed that no significant GCK mutations, the present result is also consistent with Saudi Arabia result which founded that, there is no relationship between GCK gene mutation at positions 682A>G, p.T228A and gestational diabetes women in Saudi Arabia (Hassan SM, et al. 2013) on contrast the present result disagreement with that result finding of this study indicate that mutations in GCK/MODY2 gene are a very common cause of MODY in IRAN (Javadi M, et al. 2013) and that result in also Italian population. (Mantovani V, et al. 2003) Disagreement maybe due to different habits or sample size or maybe in other exons so cannot exclude the possibility of GCK gene in the pathogenesis of MODY2 in the Sudanese populations. We need more investigation in another part of the gene

On the other hand this study observed that during analysis of the (DNA SEQUENCING) three Synonymous mutations were found, in one family that may be related to change in protein structure. That related to some studies have demonstrated that synonymous polymorphisms can affect messenger RNA splicing, stability, and structure as well as protein folding. These changes can have a

significant effect on the function of proteins, change cellular response to therapeutic targets, and often explain the different responses of individual patients to a certain medication. (Ryan Hunt, et al. 2009; Chamary, et al. 2012; Karambataki M, et al. 2014)

In conclusion, the findings of this study indicate that mutations in GCK/MODY2 gene at positions 682A>G, p.T228A was not detected in Sudanese diabetic families. The present of the synonymous mutations and the affect of messenger RNA splicing, stability, and structure as well as protein folding may be a target for more investigation and therapeutic intervention.

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