



## DETECTION OF EXON 1 HEPATOCYTE NUCLEAR FACTOR 1A MUTATIONS AND POLYMORPHISM ASSOCIATED WITH MONOGENIC DIABETES IN IRAQIS

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### ABSTRACT

Maturity Onset Diabetes of Young (MODY) is a monogenic and autosomal dominant form of diabetes mellitus with onset of the disease often before 25 years of age. It is due to dysfunction of pancreatic  $\beta$  cells characterized by non-ketotic diabetes and absence of pancreatic auto-antibodies. MODY subtype HNF1A was the most common form of MODY occurs due to mutations in the HNF1A gene on chromosome 12. This subtype was characterized by a progressive reduction in insulin secretion that may require insulin therapy, but like HNF4A subtype it was sensitive to sulfonylurea which may be very effective in controlling hyperglycemia. Our study included 63 non obese patients having early onset of non immunogenic diabetes with strong family history of diabetes Iraqis. The sequence analysis of the first exon of HNF1A gene revealed that mutations and I27L polymorphism in exon 1 of HNF1A are common cause of maturity onset diabetes of the young in the Iraqi non obese diabetic patients'.

**KEY WORD:** Maturity onset diabetes, MODY3, diabetes mellitus, hepatocyte nuclear factor 1A gene.

### INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2012). The categorical classification scheme of the disease includes the following forms: type 1 and 2 diabetes mellitus, gestational diabetes mellitus, and monogenic diabetes (Fajans *et al.*, 2001; Ogedegbe, 2006; Fajans and Bell, 2011). Monogenic  $\beta$ -cell diabetes is caused by defects of single genes critically responsible for pancreatic  $\beta$ -cell development or function, patients with monogenic  $\beta$ -cell diabetes may develop the disease since childhood, similar to type 1 diabetes (T1D), or they may develop it later in early adulthood (Sujitjooon *et al.*, 2008; Henzen, 2012). Maturity-onset diabetes of the young (MODY) represent the most common type of monogenic diabetes, it occurs due to dysfunction of pancreatic  $\beta$  cells characterised by non-ketotic diabetes and absence of pancreatic auto-antibodies and it is frequently mistaken for type 1 or type 2 diabetes mellitus (Nyunt *et al.*, 2009; Kanwal *et al.*, 2011; Attiya and Sahar, 2012; Mc Donald and Ellard, 2013). Mutations disrupting HNF1A gene (also known as TCF1 gene) are responsible for the most common subtype of monogenic diabetes, HNF1A-MODY or MODY3 leading to diabetes in the second to fourth decade of life in the absence of  $\beta$ -cell autoimmunity and insulin resistance (Ellard *et al.*, 2008, Thanabalasingham *et al.*, 2012). HNF1A was the first identified transcription factor gene to cause MODY, HNF1A is located 12q24 and encodes for a

nuclear protein that is expressed in liver, kidney,  $\beta$ -cells of the pancreas, and several other tissues (Bach *et al.*, 1992; Tronche *et al.*, 1994), contains 10 exons, and encodes a protein with 631 amino acids (Kaisaki *et al.*, 1997). HNF1A protein is composed of three functional domains: an amino terminal dimerization domain (amino acids 1-32), DNA binding domain with a homeodomain-like and POU-like motifs (amino acids 150-280) and C-terminal transactivation domain (amino acids 281- 631) (Yamagata, 2003; Sujitjooon *et al.*, 2008). In clinical practice, diagnostic differentiation between HNF1A MODY and other causes of early-onset diabetes is complicated by the overlap of phenotypic features and only minorities of cases of HNF1A-MODY are referred for definitive molecular testing (Shields *et al.*, 2010). The genetic diagnoses of HNF1A have important prognostic and therapeutic implications (Gardner and Tai, 2012).

The HNF-1A mutations including missense, nonsense, frameshift insertions/deletions, duplications, promoter region mutations, and splice site mutations are located throughout the gene. Among these, missense mutations are most common, spreading throughout the entire gene, and are concentrated in the dimerization and DNA-binding domains (Bellanne-Chantelot *et al.*, 2007). The dimerization domain (amino acids 1-32) of HNF1A protein is encoded by the first exon of HNF1A contain over than 40 previously reported mutation and polymorphisms associated maturity onset diabetes of the young (Sujitjooon *et al.*, 2008). The objective of this study is to detect the type and presence mutations and polymorphisms in the exon 1 of HNF1A causing maturity onset diabetes of the young in Iraqis.

## MATERIALS & METHODS

### Patients

Patients included in the study were 63 diabetic patients selected from the diabetic patients of the center of endocrinology and diabetes search Alrusafa/ Baghdad after taking their full acceptance, the selected subjects showed the diagnostic criteria of MODY represented by: (i) early onset of diabetes of non type 1 diabetes mellitus (usually less than 25 years), (ii) autosomal dominant inheritance and presence of diabetes in two or more generations, (iii) non obese and non-ketotic diabetes. The patients excluded from the study were the subjects who had GAD autoimmune antibodies, obese and diabetes patients without family history or diabetes caused by emotional trauma.

### Mutation screening and polymorphism

The screening for exon 1 mutations and polymorphism was done through the PCR amplification of exon 1 of the hepatocyte nuclear factor 1A of genomic DNA extracted from peripheral blood using Blood FlexiGene DNA Kit (QIAGEN) followed by DNA sequencing and analyzing.

PCR amplification of the exon 1 of the HNF 1A gene was done using the upstream and downstream primers 5' CGTGCCCTGTGGCAGCCGA -3' and 5' -GGGCTCGTTAGGAGCTGAGGG -3' (Lim *et al.* , 2008). PCR was performed in a 50 µl HF PreMix master mix (Bioneer), Primer forward 1.5 µl (10PM), Primer reverse 1.5 µl (10 PM), Template DNA 5µl, and 42 µl nuclease free distilled water. A total of 35 PCR cycles with denaturation at 94°C for 20 sec., annealing for 35 sec at 65°C and extension at 72 °C for 35 sec. were conducted. An initial DNA denaturation at 94°C was carried out for 3

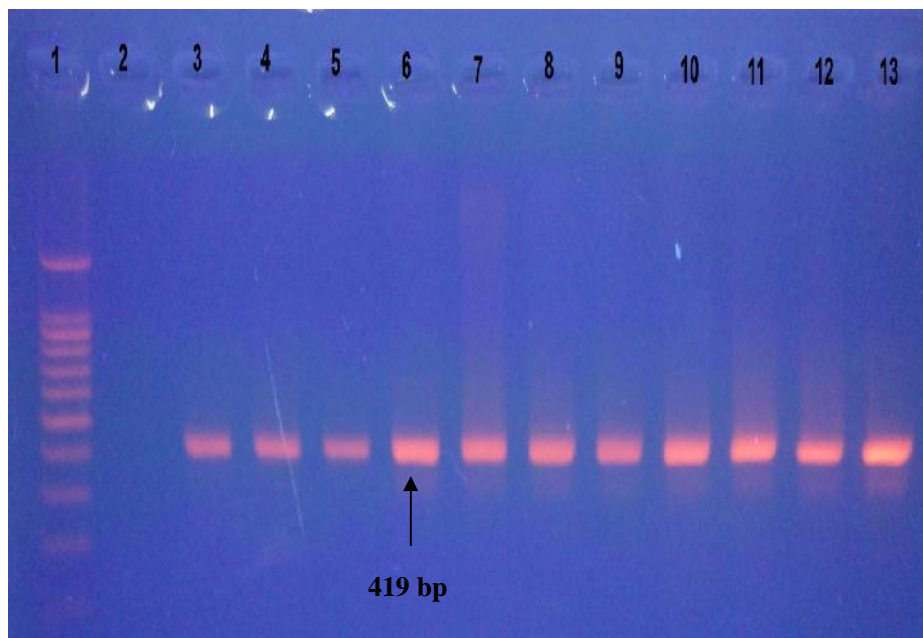
minutes and final extension at 72 °C were carried out for 5 minutes by Verti96 Thermo cyclor (Applied Biosystem). The analysis of PCR products of the HNF1A gene was done by electrophoresis on 2 % agarose gel with the use of 100 bp DNA ladder (GeneDirex) as a size marker to check the molecular weight of the amplicon. The electrophoresis was using 5 µL of the PCR product subjected to electric field power 100t for 15 min and then 40 min at 50 t. Then the gels were visualized using UV transilluminator (Vilber-Lourmat) and documented by digital camera (Canon). The PCR products were prepared for sequencing by cleaning up using Gel/PCR DNA Fragments Extraction kit (Bioneer), and sent for doing sequencing using the forward primer in Macrogen Company.

Analysis of the results of sequencing was done by using MEGA 5 program using as a reference the sequence of exon 1 of the HNF1A gene ref[NG\_011731.2] Homo sapiens HNF1 homeobox A (HNF1A), RefSeqGene.

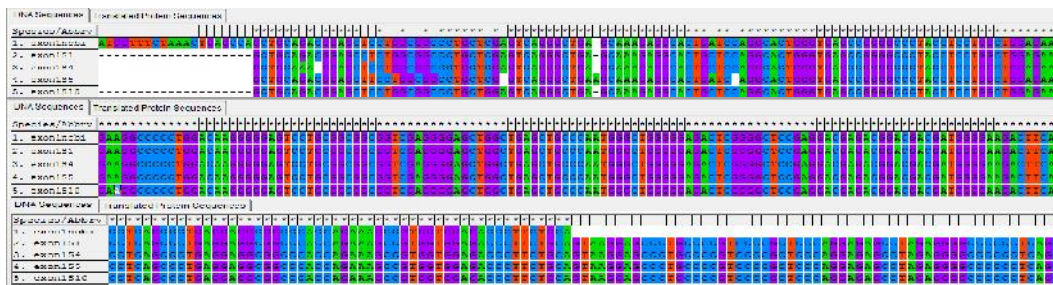
## RESULTS & DISCUSSION

The amplification of the exon 1 region of the gene gave amplicons with molecular size 419 bp (Figure 1). The amplicons were purified and sent for sequencing using the forward primer the results of sequences were analyzed using MEGA 5 program to detect the subjects having mutations or polymorphism.

The sequence analysis showed the presence of mutations causing MODY 3 in two patients and the presence of I27L polymorphism in three patients (Figure 2) the description of these mutations and polymorphism is shown in (Table 1).



**FIGURE 1:** Electrophoresis of PCR Products of Exon 1 on Agarose Gel 2% (at 100 t for 20 min and then 50 t for 40 min) Lane 1 100 bp DNA Ladder, Lane 2 -ve Control, Lane 3-13 PCR products of Exon 1



**FIGURE 2:** Sequence Analysis of Exon 1 Mutations using MEGA 5 Program

**TABLE 1:** Discription of Mutations in Exon 1

Patients	Codon	Nucleotide change	A.A. change	Designation
Patient 1	12	TC to CT	L to L	L12L
	27	A to C	I to L	I27L(Polymorphism)
Patient 4	9	G to A	Q to L	Q9L
	10	Deletion of C	Frameshift	T10fsdelC
Patient 5	27	A to C	I to L	I27L(Polymorphism)
	12	Insertion of T	Frameshift	L12fsinsT
	16	Deletion of C	Frameshift	L16fsdelC
	18	Deletion of A	Frameshift	E28fsdelA S21fsinsA
Patient 5	21	Insertion of A	Frameshift	Q28fsdelC
	28	Deletion of C	Frameshift	
	27	A to C	I to L	I27L (Polymorphism)

The I27L polymorphism found in the patients 1, 4 and 10 was one of the most common polymorphisms causing MODY 3 in different populations including both Caucasian and non Caucasian, (Chiu *et al.*, 2003; Awa, 2011; Nulli *et al.*, 2011; Bonatto *et al.*, 2012). This polymorphism occurs in the dimerization domain and causes MODY 3 in younger ages than other polymorphisms in the HNF1A gene (Awa, 2011; Nulli *et al.*, 2011). While the mutations found in patient 5 up to our knowledge have not been previously identified in other populations, but it seems that codon 12 have a mutational hot spot because there is another mutations L12H and L12F that has been previously recorded in this codon (Ryffel, 2001; Sujitjooon *et al.*, 2008).

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