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MOLECULAR INVESTIGATION OF TYPE 3 MATURITY ONSET DIABETES OF THE YOUNG CAUSED BY EXON 4 OF HEPATOCYTE NUCLEAR A1 GENE MUTATIONS IN SAMPLE OF IRAQI DIABETIC PATIENTS

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ABSTRACT
Maturity-Onset Diabetes of the Young (MODY) is a monogenic form of diabetes, consisting of a heterogeneous group of autosomal dominant inherited disorders. This study included 63 non obese patients having early onset of non immunogenic diabetes with strong family history of diabetes selected from diabetic patients attended the specialized center of endocrinology and diabetes at Alrusafa/ Baghdad during period from the begging of October 2011 till the end of May 2013.The sequence analysis of the exon 4 hepatocyte nuclear factor 1A gene reported for the first time in Iraq the presence of monogenic diabetes (maturity onset diabetes of the young type 3) as major cause of diabetes within non obese diabetic patients' early onset of non immunogenic diabetes with strong family history of diabetes.

KEY WARD: Maturity Onset Diabetes of The Young, MODY3, Diabetes mellitus, Hepatocyte Nuclear α 1 Gene.

INTRODUCTION

Maturity onset diabetes of the young (MODY) is a specific type diabetes characterized by early onset (usually before 25 years of age), autosomal dominant inheritance and usually not associated with insulin resistance (Fajans *et al.*, 2001; Scobie, 2008; Fajans and Bell, 2011). It occurs due to dysfunction of pancreatic ß cells characterized by non-ketotic diabetes and absence of pancreatic auto-antibodies (Sujjitjoon *et al*., 2008), but it is frequently mistaken for type 1 or type 2 diabetes mellitus (McCarthy and Hattersley, 2008; Shepherd, 2009; McDonald and Ellard, 2013). MODY include a group of clinically heterogeneous forms of diabetes that are defined at the molecular genetics level by mutations in different genes all show dominant inheritance and are disorders of beta cell dysfunction, but variable features include the age at onset, severity of the hyperglycaemia (Ellard *et al.*, 2008). MODYgenes includes: hepatocyte nuclear factor gene (HNF) 4A (MODY 1), glucokinase gene (MODY 2), hepatocyte nuclear factor gene HNF-1A (MODY 3), insulin promoter factor 1 (IPF-1)gene (MODY 4), HNF 1B (MODY 5), neurogenic differentiation factor 1 (NeuroD1) gene (MODY 6), Krueppel-like factor 11 (KLF11) gene (MODY 7) Carboxyl-ester lipase (CEL) gene,(MODY 8), Paired box gene 4 PAX4 (MODY 9), Insulin gene (MODY 10), and Tyrosine-protein kinase (BLK) gene (MODY 11) (Nyunt *et al*., 2009; Kanwal *et al.*, 2011). All of MODY genes are expressed in beta cells, and mutation of any of them leads to beta-cell dysfunction and diabetes mellitus (Winter, 2000). It has been shown that mutations in the HNF-1A gene (resulting in MODY 3) are the most severe and the most common cause of MODY in most populations. (Ellard, 2000, Vaxillaire and Froguel, 2008). For the mentioned reasons and the absence of any previous genetic or clinical studies about Maturity onset diabetes of the young type 3 (MODY3) or any other types of monogenic diabetes in Iraq, this study is planned to detect the presence of mutations in the exon 4 of hepatocyte nuclear factor gene that are responsible for MODY 3 the most common type of MODY in an attempt to discriminate MODY 3 patients from other Iraqi diabetic patients.

MATERIALS & METHODS

The study was carried out from October 2011 till May 2013, in Institute of Genetic Engineering and Biotechnology for Higher Studies, University of Baghdad on 63 diabetic subjects selected from the patients of the center of endocrinology and diabetes search Alrusafa/ Baghdad after taking their their full acceptance, the selected subjects showed the diagnostic criteria of MODY represented by: (i) early onset of diabetes (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 exclusion criteria of the subjects from the study were presence of autoimmune antibodies, obesity, diabetes without family history and diabetes caused by emotional trauma. Mutations detection was done through the PCR amplification of exon 4 of the hepatocyte nuclear factor A1 of genomic DNA extracted from peripheral blood using Blood FlexiGene DNA Kit (QIAGENE) followed by DNA sequencing and analyzing. PCR amplification of the exon 4 of the HNF A1 gene was done using the forward primer, 5′ CAGAACCCTCCCCTTCATGCC -3′ and the reverse primer, 5′- CCCACCTTCCCACGTGTCCC -3′ (Lehto *et al.*, 1999). PCR was performed in a 50µl AccuPower® ProFi Taq PCR PreMix master mix

(Bioneer), Primer forward 2µl (10PM), Primer reverse 1.6 µl (10 PM), Template DNA 5µl, and 41.4 µl nuclease free distilled water. A total of 38 PCR cycles with denaturation at 94°C for 20 sec., annealing for 40 sec at 65 °C and extension at 72 °C for 40 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 cycler (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 100 volt for 15 min and then 40 min at 50 volt. Then the gels were visualized using UV transilluminator and documented by digital camera. The PCR products were prepared for sequencing by cleaning up using Gel/PCR DNA Fragments Extraction kit (Bioneer), and were kept frozen and sent to *El Weratha Company* Genetics Company (Biotechnology Products and Services) Amman/Jordan for doing sequencing for the forword primers in Macrogen company. Analysis of the results of sequencing was done by using the computural programs BIOEDIT and MEGA 5 to detect the presence of the mutations and their effect on the protein levels with the use of references sequences of exon 4 of the HNF1A gene ref|NG_011731.2| Homo sapiens HNF1 homeobox A (HNF1A), RefSeqGene.

RESULTS & DISCUSSION

The PCR amplification of exon 4 gave amplicones with molecular size 360 bp (Figure 1).

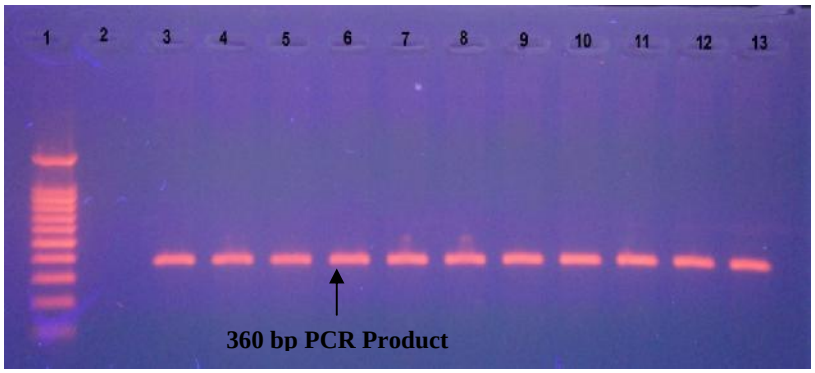

FIGURE 1: Electrophoresis of PCR Products of Exon 4 on Agarose Gel 2% (at 6.666 V/ cm for 20 min and then 3.333 V/ cm for 40 min)
Lane 1 100 bp DNA Ladder, Lane 2 –ve Control (with out DNA), Lane 3-13 PCR products of Exon 4

The remaining PCR products of exon 4 were cleaned and sent for sequencing, then the results of sequencing were analyzed using BioEdit and MEGA 5 programs detect the patients having mutations in exon 4 of hnf 1 α gene. The analysis revealed that nine patients had these mutations (Figure 2, Table 1).

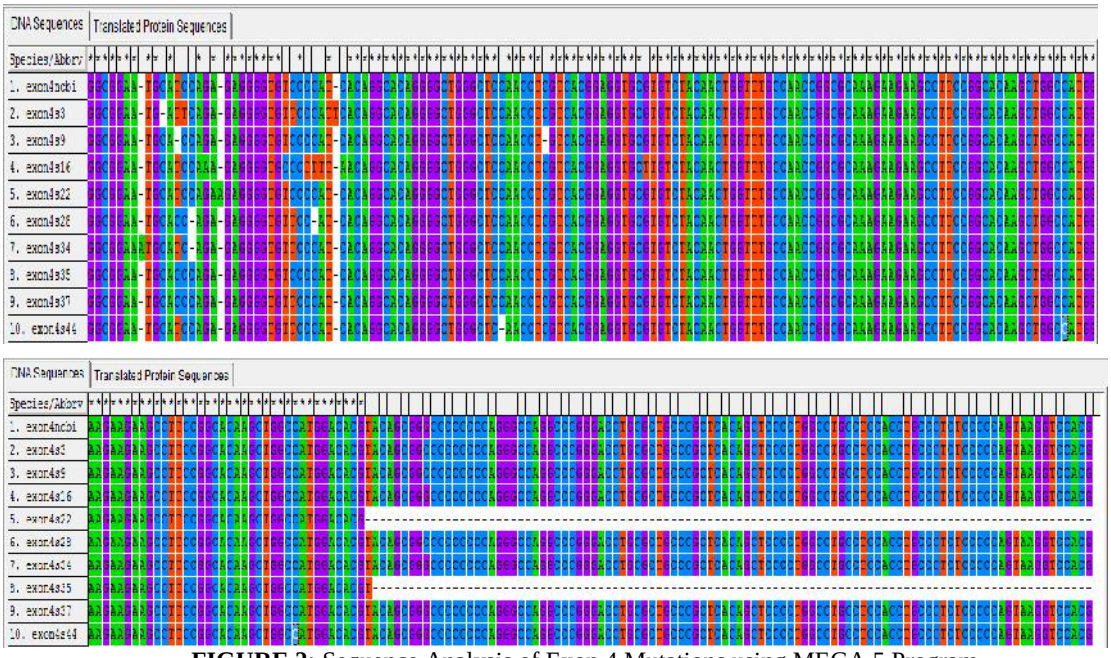

FIGURE 2: Sequence Analysis of Exon 4 Mutations using MEGA 5 Program

TABLE 1: Discription of Mutations in Exon 4

Patients	Codon	Nucleotide change	A.A. change	Designation	Notes
Patient 3	241	Deletion of C	Frameshift	C241fsdelC	Mutation
	242	C to T	I to I	I242I	
	249	Insertion of T	Frameshift	S249fsinsC	
Patient 9	242	Deletion of T	Frameshift	I242fsdelT	Mutation
	258	Deletion of C	Frameshift	L258fsdelC	
Patient 16	243	G to A	Q to Q	Q243Q	
	247	T to C	S to P	S247P	
	248	CCA to TTT	P to F	P248F	
	263	G to T	R to L	R 263L	STOP
	249	C to A	S to STOP	STOP	CODON
Patient 22	244	Insertion of A	Frameshift	R244fsinsA	Mutation
Patient 28	242	Deletion of T	Frameshift	I242fsdelT	Mutation
	247	Deletion of C	Frameshift	S247fsdelC	
Patient 34	240	Insertion of A	Frameshift	E240fsinsA	Mutation
	243	Deletion of C	Frameshift	Q247fsdelC	
	249	C to T	S to F	S249F	
Patient 35	242	T to C	I to T	I242T	
Patient 37	247	C to T	S to F	S247F	
Patient 44	247	C to T	S to F	S247F	Mutation
	256	Deletion of C	Frameshift	S256fsdelC	

All the mutations within exon 4 in the Iraqi diabetic patients have not been previously identified in other populations except the mutation S249F in patient 34 which had been previously identified in Poland (Skupien *et al.*, 2008), but codons 241 have two other mutations (C241G, C241R) (Ryffel, 2001) and codon 263 also have two other mutations (R263H, R263C), the first one recorded in India and the second one in Japan (Tonooka *et al.*, 2002; Radha *et al.*, 2009) also codon 240 which have mutation in patient 34 have other mutation E240Q (Kim *et al*., 2003).

REFERENCES

Ellard, S. (2000) Hepatocyte nuclear factor 1 alpha (HNF-1 alpha) mutations in maturity-onset diabetes of the young. *Human Mutation*. Vol. 16:377-385.

Ellard, S., Bellanne-Chantelot, C., Hattersley, A.T. (2008) Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. *Diabetologia*. Vol.51:54 -553.

Fajans, S.S., Bell, G.I., Polonsky, K.S. (2001) Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med*. Vol.345:971-980.

Fajans, S. S. and Bell, G. (2011) MODY: History, genetics, pathophysiology, and clinical decision making. *Diabetes Care*. Vol. 34: 1878-1884.

Kanwal, A., Fazal, S., Ismail, M. and Naureen, N. (2011) A narrative insight to maturity onset diabetes of the young. *Clinical Reviews and Opinion*. Vol.3: 6-13.

Kim, S., Ma, X., Klupa, T., Powers, C., Pezzolesi, M., Warram, J.H., Rich, S.S., Krolewski, A.S., and Doria, A. (2003) Genetic modifiers of the age at diagnosis of diabetes (MODY3) in carriers of hepatocyte nuclear factor-1α mutations map to chromosomes 5p15, 9q22, and 14q24. *Diabetes*. Vol. 52:2182-2186.

Kupien, J., Gorczynska-Kosiorz, S., Klupa, T., Cygnek, K., Wanic, K., Borowiec, M., Sieradzki, J. and Malecki, M.T. (2008) Molecular background and clinical characteristics of HNF1A MODY in Polish population. *Diabetes and Metabolism*. Vol. 34: 524-528.

Lehto, M., Wipemo, C., Ivarsson, S.A., Lindgren, C., Lipsanen-Nyman, M., Weng, J., Wibell, L., Widen, E., Tuomi, T. and Groop, L. (1999) High frequency of mutations in MODY and mitochondrial genes in Scandinavian patients with familial early-onset diabetes. *Diabetologia*. Vol. 42: 1131-1137.

McCarthy, M.I. and Hattersley, A.T. (2008) Learning from molecular genetics: Novel insights arising from the definition of genes for monogenic and type 2 diabetes. *Diabetes*. Vol. 57: 2889-2898.

McDonald T. J. and Ellard, S. (2013) Maturity onset diabetes of the young: identification and diagnosis. *Annals of Clinical Biochemistry*. Vol. 50: 403-415.

Nyunt, O., Wu, J.Y., McGown, I.N., Harris, M., Huynh, T., Leong, G.M., Cowley, D.M. and Cotterill, A.M. (2009) Investigating Maturity Onset Diabetes of the Young. *Clin Biochem Rev*. Vol 30: 67-74.

Radha, V. Ek, J. Anuradha, S. Hansen, T. Pedersen, O. and Mohan, V. (2009) Identification of Novel Variants in the Hepatocyte Nuclear Factor-1α Gene in South Indian Patients with Maturity Onset Diabetes of Young. *Journal of Clinical Endocrinology & Metabolism*. Vol. 94: 1959-1965.

Ryffel, G. U. (2001) Mutations in the human genes encoding the transcription factors of the hepatocyte

nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. *Journal of Molecular Endocrinology*. Vol. 27: 11-29.

Scobie, I. N. (2008) Atlas of diabetes mellitus. Third Edition. Informa UK Ltd.

Shepherd, M. (2009) Genetic testing clarifies diagnosis and treatment in a family with both HNF1A and type 1 diabetes. *Pract Diab*. Vol. 26:269-273.

Sujjitjoon, J., Jungtrakoon, P., Boonyasrisawat, W., Chongjaroen, N., Chukijrungroat, T., Kooptiwut, S., Plengvidhya, N., Banchuin, N.and Yenchitsomanus, P. (2008) Molecular genetics of monogenetic beta-cell diabetes. *Thai Journal of Genetics*. Vol. 1: 93-108.

Tonooka, N., Tomura, H., Takahashi, Y., Onigata, K., Kikuchi, N., Horikawa, Y.,Mori, M. and Takeda, J. (2002) High frequency of mutations in the HNF-1α gene (TCF1) in non-obese patients with diabetes of youth in Japanese and identification of a case of digenic inheritance. *Diabetologia*. Vol. 45:1709-1712.

Vaxillaire, M. and Froguel, P. (2008) Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. *Endocrine Reviews*. Vol. 29: 254-264.

Winter, W.E. (2000) Molecular and biochemical analysis of the MODY syndromes. *Pediatric Diabetes*. Vol.1: 88-117.