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Correlation between gene polymorphism rs7903146 and diabetes mellitus In Iraqi population

A Research

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BY

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا

مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ

الْحَكِيمُ)

صدق الله العظيم

سورة البقرة: الآية 32

Dedication

To who was present with me at all times in my heart
and mind to prophet of peace...

“Mohammed”

Peace and prayer be on him and his purified family.

To my great family who encouraged me

To all my friends

Zahraa Ali Mohammed and Zahraa Ali Mahdi

Acknowledgments

First of all, praise is to “Allah”, who enabled us to overcome all the difficulties that were associated with this work till I brought it to the present state. Peace and prayer be on the most honorable Mohammed and his purified family.

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1. Introduction

Diabetes mellitus (DM) is a serious medical problem affecting millions of peoples worldwide, and has a great socio-economic impacts(1).

The prevalence of type 2 diabetes (T2D) is increasing in an alarming rate(2-4) Population ageing and obesity are considered the main causes of diabetes (5). Although the risk of developing the disease is also strongly influenced by inheritance in which Genetic susceptibility to T2D is believed to be polygenic (6, 7)as it has been indentified transcription factor-7-like 2 (TCF7/L2) as susceptible gene (8, 9).Genetic variants in the gene encoding this transcription factor have been consistently associated with T2D and impaired insulin secretion Among them, the rs7903146 T allele is probably the best marker to evaluate the effect of this gene on T2D risk(4).

This allele increases the risk of T2D by 1.45 in heterozygous and by 2.41 in homozygous .All these findings have been replicated in populations of different ethnic descent including caucasian Europeans Japanese and Indian people ,Latin Americans and West Africans (6, 10, 11) , representing the main ethnical groups with a high prevalence of T2D(12).

ELMO1 is a soluble cytoplasmic protein that functionally cooperates with CRKII and dedicator of cytokinesis 180 (DOCK180) to mediate cytoskeletal rearrangements during phagocytosis of apoptotic cells and cell motility in mammalian cells (Gumienny et al., 2001). Functional studies of ELMO1

expression reveal that increased levels were observed in COS cells cultured at high glucose concentration, loss of cell adhesion properties and enhanced synthesis of collagen and fibronectin in ELMO1 transfected cells suggest a pathological role in kidney disease (Shimazaki et al., 2005; Shimazaki

et al., 2006). This study aims at exploring any possible correlation between diabetes mellitus and gene polymorphism rs7903146.

2. Materials and Methods

2.1 Materials

2.1.1 Equipment and Apparatus

Different equipment and apparatuses have been used throughout the study as shown in table 1

Table 1: Equipment used in the study

Equipment	Company
Autoclave	Gallenkamp (England)
Cooled centrifuge	Labnet (USA)
Thermocycler	Labnet (USA)
Distillatory unit	Kent (England)
Hot plate magnetic stirrer	Stuart scientific (U.K.)
Sensitive balance	Sartorius (Germany)
Spectrophotometer	Labnet(USA)
Vortex mixer	Buchi (Switzerland)
DNA –Gel Electrophoresis	Labnet (USA)
Micropipettes	Witeg (USA)

Gel documentation system	Labnet (USA)
Water bath	Labnet (USA)

2.1.2 Chemicals and buffers

2.1.2.1 TBE buffer 5X (Maniatis *et al.*, 1982)

It is composed of:-

Tris-Base 54 g
 Boric acid 27.5 gm
 EDTA 0.5M (pH 8) 20 ml

The volume was brought to up 1 L and autoclaved

2.1.2.2 Ethidium bromide solution (10 mg/ml) (Maniatis *et al.*, 1982)

Ethidium bromide (0.1 g) was dissolved in 10 ml of D.W and stirred with a magnetic stirrer for six hours to ensure the complete dissolving, then it filtrated and stored in a dark bottle, wrapped with aluminum foil at 4°C.

2.1.2.3 Agarose gel

Agarose 1% concentration was used, dissolved in TBE 1X using hotplate.

2.2 Methods

2.2.1 Study individuals

This study was carried out on 40 Iraqi individuals, aged between (14-69) years represented by 20 patients who admitted to Al-Diwaniyha teaching hospital and 20 healthy controls. Blood samples were collected from subjects. About three milliliters of blood withdrawal from each subject and placed into Ethylenediaminetetraacetic acid (EDTA)-tubes then transferred to the laboratory in cooling conditions in less than one hour.

2.2.2 DNA Extraction

DNA was isolated from peripheral blood by means of FavorPrep Blood Genomic DNA Extraction Mini Kit (South Korea) according to the manufacturer's instructions at Department of Medical Biotechnology / College of Biotechnology / University of Al-Qadisiyha and stored at -20 °C for Polymerase Chain reaction.

2.2.3 Genotyping

Genotyping was took place on cycler machine (LABNET) using primers table (2). Amplification conditions were 35 cycles of 94°C / four minutes, 94°C / 30 seconds, 63°C / one minute, and 72°C / two minutes with a final extension step of 72°C / eight minutes, PCR products were run on 1.8% agarose gel and stained with Ethidium bromide then analyzed using UV transilluminator, standard DNA ladder 100bp (Bioneer, South Korea) was used.

DNA amplifications were repeated three times using the same conditions to confirm the results with negative controls. Product sizes in order to check the variant rs1345365 polymorphism are 629 bp for two outer primers (control bands), 284 bp for G allele and 461 bp for A allele according to (13).

Table (2): Specific primers applied for polymorphism determination of ELMO1 gene

Primer name	Sequence
Forward inner	5' GCCACTTCTTCCCCTACAACATTGA 3'
Reverse inner	5' GCCAGTGAGAGAGTAATACTATTACGTTTC3'
Forward outer	5' TGCCATAGGTACTGCTTCTCTGAGT 3'
Reverse outer	5' CTGAAGTCTAGTAAGAGCTCAAGGTCAGT 3'

3. Results and discussion

Specific primers were used to in an attempt to detect a possible genetic polymorphism between patients diagnosed with identified with diabetes mellitus in Al-Qadisiyha. There are many scientific methods and protocols were applying by many researcher and laboratory workers to detect and analyze the mutations that are related to diabetes mellitus type 2 . Tetra-ARMS PCR technique is considered as one of the best technique due to its easy and inexpensive to be compared with the other technique in which we prefer to use it in this study.

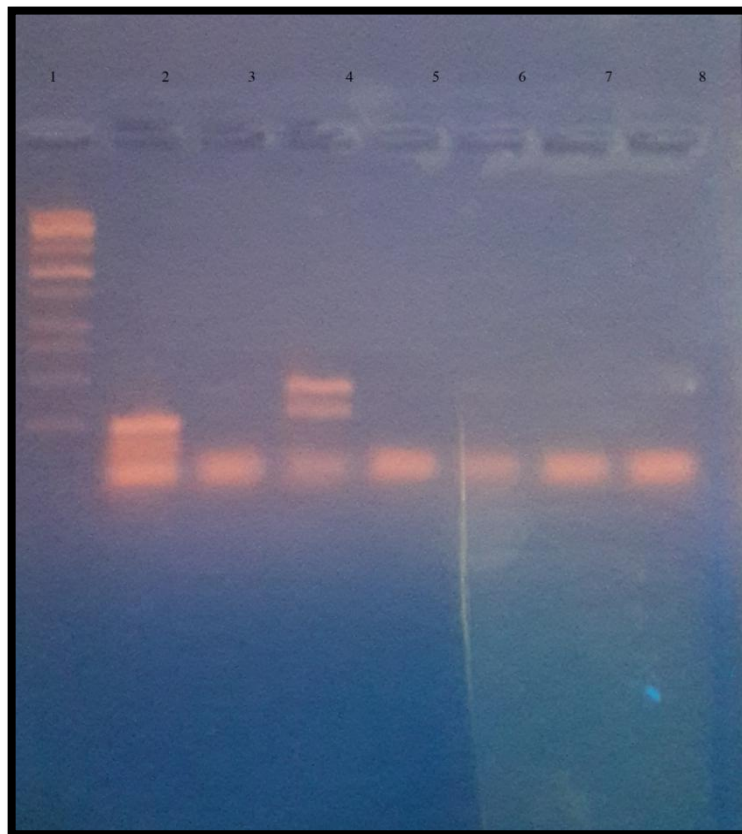


Figure 1 : 1% Agarose electrophoresis of PCR product 5v/cm, lane1, DNA ladder 100 bp; lane2-3, patients; lane 4-8: healthy individuals

Tetra-ARMS PCR technique that applied in this study revealed presence of single nucleotide polymorphism figure (1-2)

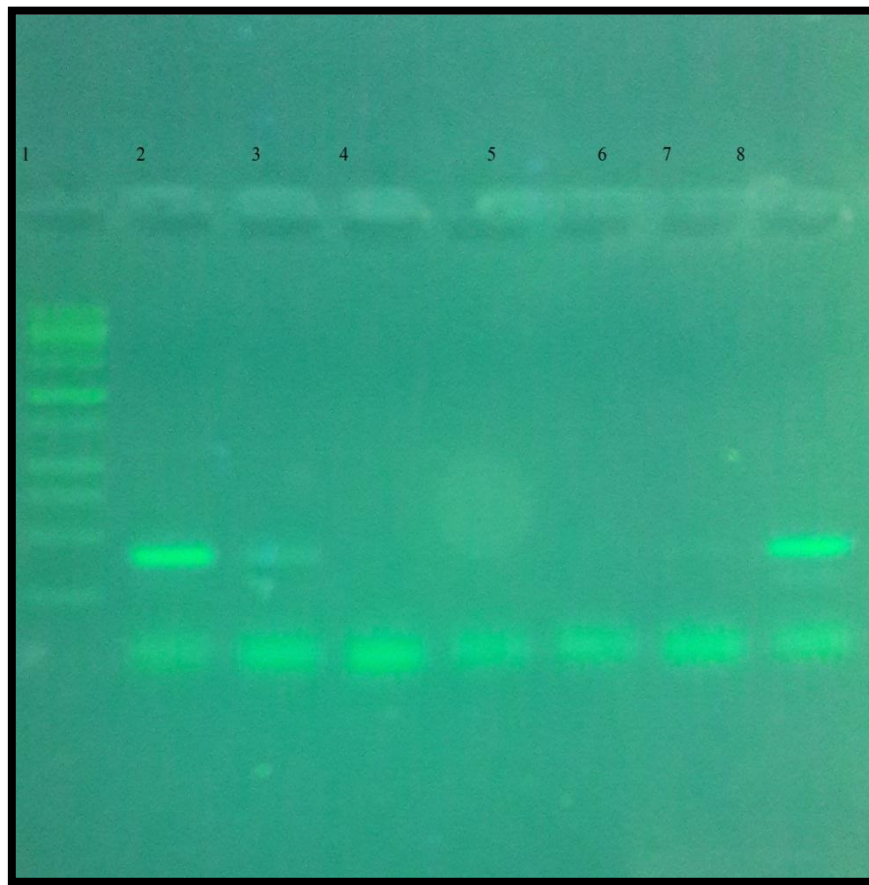


Figure 2: 1% Agarose electrophoresis of PCR product 5v/cm, lane1, DNA ladder 100 bp; lane2-3, patients; lane 4-8: healthy individuals

ELMO1 is a soluble cytoplasmic protein that functionally cooperates with CRKII and dedicator of cytokinesis 180 (DOCK180) to mediate cytoskeletal rearrangements during phagocytosis of apoptotic cells and cell motility in mammalian cells (14).

The results showed the allelic and genotyping of the individuals covered by this study as it appears in table (3).

Table 3: rs7903146 genotypes in patients and healthy individuals

SNP ID	Allele/Genotype	Patients	Healthy
rs1345365		N(%)	N(%)
	A	8 (40)	9 (45)
	G	9 (45)	9 (45)
	AA	2 (10)	1 (5)
	AG	1 (5)	1 (5)
	GG	0	0

In which allele “A” represented 40 % in patients and 45% in healthy individuals ; while allele “G” genotyped with same percentage for both patients and healthy individuals ; on other hand, combined allelic genotypes of “AA” showed 10% , 5% for patients and healthy persons in successive. “AG” registred with 1% for both study groups , while “GG” allelic genotype typed with 0%.Our results are similar to results of (10, 13) in which it seems that ELMO1 plays an important role in the development of type 1 diabetic nephropathy (11) which agrees to the results of our study.

4. Conclusion

We concluded from our study that rs7903146 polymorphism was genotyped in patients with diabetes mellitus in Iraq.

Author’s notes

The manuscript has written by Endnote X7.

5. References

1. Abdel-Moneim A, Bakery HH, Allam G. The potential pathogenic role of IL-17/Th17 cells in both type 1 and type 2 diabetes mellitus. *Biomed Pharmacother.* 2018;101:287-92.
2. Wu HY, Wang Y, Chen M, Zhang X, Wang D, Pan Y, et al. Association of ELMO1 gene polymorphisms with diabetic nephropathy in Chinese population. *J Endocrinol Invest.* 2013;36(5):298-302.
3. Turki A, Mzoughi S, Mtitaoui N, Khairallah M, Marmouch H, Hammami S, et al. Gender differences in the association of ELMO1 genetic variants with type 2 diabetes in Tunisian Arabs. *J Endocrinol Invest.* 2018;41(3):285-91.
4. Shimazaki A, Kawamura Y, Kanazawa A, Sekine A, Saito S, Tsunoda T, et al. Genetic variations in the gene encoding ELMO1 are associated with susceptibility to diabetic nephropathy. *Diabetes.* 2005;54(4):1171-8.
5. Akoumianakis I, Antoniadou C. Impaired Vascular Redox Signaling in the Vascular Complications of Obesity and Diabetes Mellitus. *Antioxid Redox Signal.* 2018.
6. Hanson RL, Millis MP, Young NJ, Kobes S, Nelson RG, Knowler WC, et al. ELMO1 variants and susceptibility to diabetic nephropathy in American Indians. *Mol Genet Metab.* 2010;101(4):383-90.
7. Yang C, Sorokin A. Upregulation of fibronectin expression by COX-2 is mediated by interaction with ELMO1. *Cell Signal.* 2011;23(1):99-104.
8. Pirini F, Noazin S, Jahuira-Arias MH, Rodriguez-Torres S, Friess L, Michailidi C, et al. Early detection of gastric cancer using global, genome-wide and IRF4, ELMO1, CLIP4 and MSC DNA methylation in endoscopic biopsies. *Oncotarget.* 2017;8(24):38501-16.
9. Schaker K, Bartsch S, Patry C, Stoll SJ, Hillebrands JL, Wieland T, et al. The bipartite rac1 Guanine nucleotide exchange factor engulphment and cell motility 1/dedicator of cytokinesis 180 (elmo1/dock180) protects endothelial cells from apoptosis in blood vessel development. *J Biol Chem.* 2015;290(10):6408-18.
10. Bodhini D, Chidambaram M, Liju S, Revathi B, Laasya D, Sathish N, et al. Association of rs11643718 SLC12A3 and rs741301 ELMO1 Variants with Diabetic Nephropathy in South Indian Population. *Ann Hum Genet.* 2016;80(6):336-41.
11. Hathaway CK, Chang AS, Grant R, Kim HS, Madden VJ, Bagnell CR, Jr., et al. High Elmo1 expression aggravates and low Elmo1 expression prevents diabetic nephropathy. *Proc Natl Acad Sci U S A.* 2016;113(8):2218-22.

12. Leak TS, Perlegas PS, Smith SG, Keene KL, Hicks PJ, Langefeld CD, et al. Variants in intron 13 of the ELMO1 gene are associated with diabetic nephropathy in African Americans. *Ann Hum Genet.* 2009;73(2):152-9.
13. Mehrabzadeh M, Pasalar P, Karimi M, Abdollahi M, Daneshpour M, Asadolahpour E, et al. Association between ELMO1 gene polymorphisms and diabetic nephropathy in an Iranian population. *J Diabetes Metab Disord.* 2015;15:43.
14. Komander D, Patel M, Laurin M, Fradet N, Pelletier A, Barford D, et al. An alpha-helical extension of the ELMO1 pleckstrin homology domain mediates direct interaction to DOCK180 and is critical in Rac signaling. *Mol Biol Cell.* 2008;19(11):4837-51.

Author's notes

The manuscript of paper was done by Endnote X7.8.